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THE RELATION BETWEEN THE PROTEIN CONTENT OF WESTERN CANADIAN HARD RED SPRING WHEAT, AMBER DURUM WHEAT, AND BARLEY[1]

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The importance of protein content as an index of the strength of bread wheats, particularly within any one class, such as the hard red spring or the hard red winter wheats, has been established by numerous researches and is widely recognized. The value of durum wheats for macaroni-making purposes is chiefly determined by their ability to yield macaroni possessing a brilliant intense yellow colour, and studies recently conducted in this laboratory indicate that, aside from the concentration of yellow pigments present, the protein level materially influences the visual appearance. There is some evidence that macaroni processed from durum semolina of low protein content is deficient in translucency; moreover, in the manufacture of "long-goods" it is necessary to maintain the protein content above a more or less definite minimum, in order to prevent undue stretching during the drying or curing process. On the other hand, high-protein semolina frequently yields macaroni possessing a greyish cast.

In malting barley, protein is also important, since within varieties it is inversely correlated with malt extract and directly correlated with diastatic activity. Thus barleys of lower than average protein content are generally preferred for brewers' malts, whereas those of somewhat higher protein content serve well for the production of the diastatic malts used in the distilling and malt extract industries.

It is well known that the chemical composition of the cereal grains is materially influenced by the environment in which they are grown. Extensive studies with wheat have shown that climatic and weather conditions, particularly during the post-floral period, are of paramount importance. Short ripening periods favour the production of grain of high protein content. Thus, high temperatures and the absence of excessive moisture, which hasten maturation, result in high-protein wheat, whereas cool summers with high rainfall and a relatively long growth period are conducive to the production of low protein grain. Since rainfall exerts its effect through variations in available soil moisture, it is not surprising that wheat grown under irrigation is of lower protein content than when grown on non-irrigated land.

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In regard to soil, texture is more important than chemical composition, since upon it depends the soil's capacity to hold water. Moisture retentive soils with relatively high coefficients of availability of moisture produce wheats of lower protein content than soils with low coefficients. While the physical properties of the soil have been found to have a greater influence on protein content than its chemical composition, numerous experiments have shown that, other factors being equal, an increase in the protein content of wheat results from increasing the "available nitrogen" in the soil. Thus, under similar climatic conditions, wheat grown on summer-fallow or after legumes is of higher protein content than wheat which is grown after cereal grains or grasses. Similarly, the application of inorganic forms of nitrogenous fertilizers usually increases the protein content somewhat but the increase is generally considerably less than that resulting from the use of legumes in the crop rotation. Experiments reported in the literature show that phosphates applied in liberal amounts generally lower the protein content slightly while potash does not materially affect the composition of the grain.

In the extensive wheat-producing areas of Western Canada, the crop is grown under a wide range of soil and climatic conditions, and the milling industry has long realized that wheat grown in the prairie soils in the southern and central districts of Manitoba, Saskatchewan and Alberta averages higher in protein content and baking strength than that grown in the park and wooded belts of the north, where the available moisture is higher and the rate of ripening is slower. It is also recognized in a general way that in addition to the effects of average climatic differences between widely separated regions, the protein content of wheat from any one locality is influenced by annual fluctuations in the weather conditions.

TABLE 1.—NUMBER OF SHIPPING POINTS FROM WHICH SPRING AND DURUM WHEATS, AND SPRING WHEAT AND BARLEY, WERE COLLECTED IN EACH YEAR

Province	Number of shipping points					
	Hard red spring wheat and barley			Hard red spring and amber durum wheat		
	1934	1936	1937	1934	1936	1937
Manitoba	122	120	117	109	92	73
Saskatchewan	43	77	4	22	56	1
Alberta	4	17	6	—	—	—
Total	169	214	127	131	148	74

TABLE 2.—AVERAGE NUMBER OF SAMPLES REPRESENTED IN MEAN PROTEIN VALUES FOR SHIPPING POINTS

Year	Hard red spring	Barley	Hard red spring	Amber durum
1934	5.1	3.6	5.1	4.7
1936	4.5	6.8	5.8	4.2
1937	4.1	4.3	3.6	5.4

In order to obtain more detailed information on the potentialities of the different localities in Western Canada for the production of wheats of various protein levels, annual surveys of the protein content of the higher grades of hard red spring wheat, from large numbers of shipping points, were instituted by this laboratory in 1927. In 1934, similar protein surveys of amber durum wheat and barley were also started. The results of each survey, classified according to province, shipping point and grade, have been issued annually in mimeographed form, together with a coloured map on which the areas producing wheat of different protein content are designated in a general way by different colours.

A rough comparison of the maps for the three cereals in any particular year showed that areas yielding high-protein hard red spring wheat also tend to yield high-protein durum wheat and barley, and that a similar situation exists with respect to low protein areas. It thus appears that environmental conditions have a similar influence on the protein content of all three cereals. Accordingly, a statistical study was undertaken to determine whether the hard red spring wheat data could be employed for zoning, according to protein level, the barley and durum wheat growing areas of the Prairie Provinces, thus dispensing with the necessity of conducting separate protein surveys for durum wheat and barley.

RESULTS AND DISCUSSION

For the statistical studies, the protein surveys of the three cereals for the years 1934, 1936 and 1937 were utilized. The 1935 data were not used since, owing to unfavourable conditions, including drought and wheat stem rust, there were only 27 shipping points in 1935 for which both hard red spring and durum wheat protein data were available, and only 22 stations for hard red spring wheat and barley.

The mean protein content of hard red spring wheat grading Nos. 1 Hard and 1 Northern in 1934 and Nos. 1 Hard, 1 Northern and 2 Northern in 1936 and 1937 was computed for each shipping point common to durum wheat and barley; in 1934, grade No. 2 Northern was not used, since the Garnet variety was eligible for this grade at that time, and it is known that this variety tends to produce wheat of lower protein content than the other varieties eligible for the top grades. Similarly, the mean protein values for the principal malting barley grade No. 3 Extra C.W. Six-Row and for amber durum wheat, grades Nos. 1 C.W., 2 C.W., and 3 C.W., inclusive, were computed for each shipping point. Shipping points which were represented by only one sample of any of the three cereals were eliminated, as it was felt that a single value was not a sufficiently reliable index of the mean protein content for a shipping point. The distribution of the shipping points represented is shown geographically in Figures 1 and 2, and Table 1 summarizes them according to provinces. The average number of samples of each cereal involved in computing the respective mean protein values for each shipping point is shown in Table 2. Ranges, means, standard deviations and coefficients of variability for each year, are given in Table 3. The relationships between the mean protein contents of hard red spring wheat and barley, and of hard red spring and amber durum wheats, are shown graphically in the scatter diagrams given in Figures 3 and 4.

Reference to Table 3 will show that the mean protein content of barley was lower than that of hard red spring wheat by 2.1% in 1934, by 0.7% in 1936, and by 1.1% in 1937. The protein ranges for barley also tended to be correspondingly lower. On the other hand, both ranges and mean values for amber durum wheat are reasonably close to those for spring wheat, though the mean protein content of the former crop was

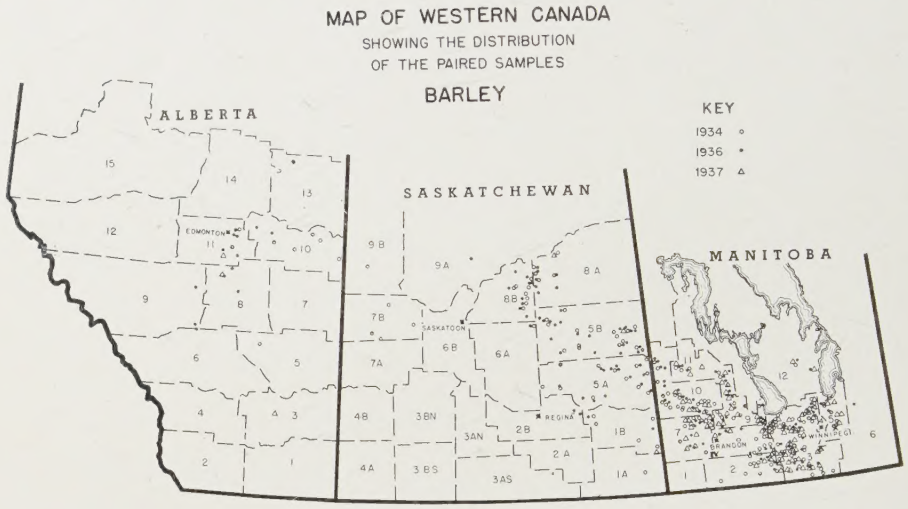

FIGURE 1. Distribution of stations, by province and crop-reporting district, shipping both hard red spring wheat and malting barley.

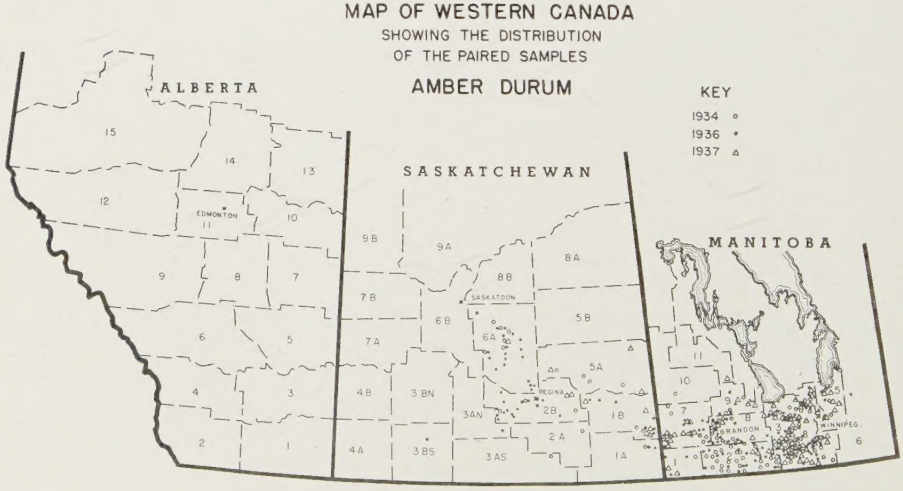

FIGURE 2. Distribution of stations, by province and crop-reporting district, shipping both hard red spring and amber durum wheats.

0.6% lower than that of the latter in 1937. These data thus fail to support the general beliefs that durum wheat has a higher protein content than hard red spring wheat grown in the same district, and that the protein content of durum wheat is more susceptible to changes in environmental conditions than that of hard red spring wheat.

The correlation and regression coefficients for each year, for spring wheat and barley, and for spring and durum wheats, are shown in Table 4. All correlation coefficients are significant but none is particularly high. Considerable differences exist within each set of regression coefficients. In these circumstances, it seemed advisable to examine the inter-annual homogeneity of the relations between each pair of grains by means of an analysis of residual variance. The resulting statistics are given in Table 5.

The statistics may be conveniently considered in relation to the equations which might be used for predicting barley or durum wheat protein from red spring wheat protein. These apparently take the form of an

TABLE 3.—STATISTICAL CONSTANTS FOR MEAN PROTEIN DATA[1] FOR SHIPPING POINTS

Year	Number of stations	Range				Means		Standard deviations		Coefficients of variability	
		Red spring		Other crop		Red spring	Other crop	Red spring	Other crop	Red spring	Other crop
		%	%	%	%	%	%	%	%	%	%
		Barley and hard red spring wheat									
1934	169	10.8 – 16.8		8.1 – 14.0		13.7	11.6	1.10	1.08	8.0	9.3
1936	214	10.2 – 17.6		10.0 – 15.3		14.5	13.8	1.33	1.10	9.2	8.0
1937	127	10.2 – 15.7		9.4 – 14.7		13.5	12.4	1.03	0.83	7.6	6.7
All years	510	10.2 – 17.6		8.1 – 15.3		14.0	12.3	1.26	1.13	9.0	9.2
		Amber durum and hard red spring wheat									
1934	131	12.2 – 16.2		11.1 – 16.8		14.2	14.0	0.81	1.12	5.7	7.9
1936	148	12.5 – 17.6		11.8 – 18.2		15.5	15.5	1.16	1.41	7.5	9.1
1937	74	11.1 – 16.0		11.2 – 15.8		13.8	13.2	1.01	1.10	7.3	8.4
All years	353	11.1 – 17.6		11.1 – 18.2		14.7	14.4	1.23	1.55	8.4	10.8

[1] In computing protein content, the factor 6.25 was used for barley, and the factor 5.7 for red spring and amber durum wheats. All results are expressed on a 13.5% moisture basis.

TABLE 4.—CORRELATION AND REGRESSION COEFFICIENTS

Year	Barley and red spring wheat			Amber durum and red spring wheat		
	No. of samples	Correlation coefficient	Regression coefficient	No. of samples	Correlation coefficient	Regression coefficient
1934	169	.55**	.54	131	.55**	.77
1936	214	.74**	.62	148	.76**	.92
1937	127	.55**	.44	74	.63**	.68
Total within years	510	.65**	.56	353	.68**	.84

** Significance exceeds the 1% level.

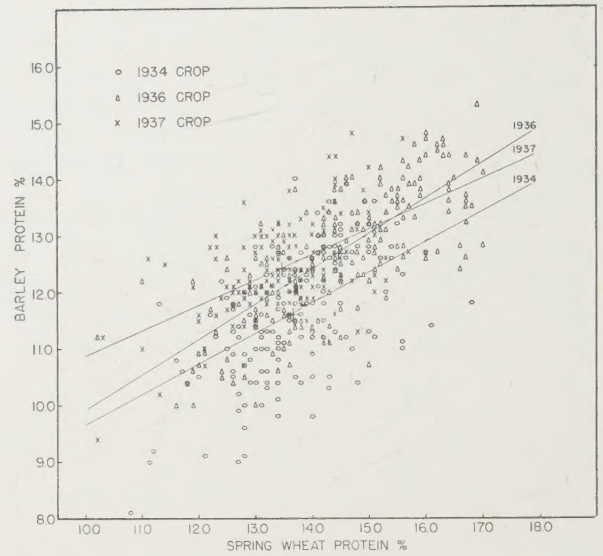

FIGURE 3. Scatter diagram and regressions for mean protein contents of hard red spring wheat and malting barley from corresponding shipping points.

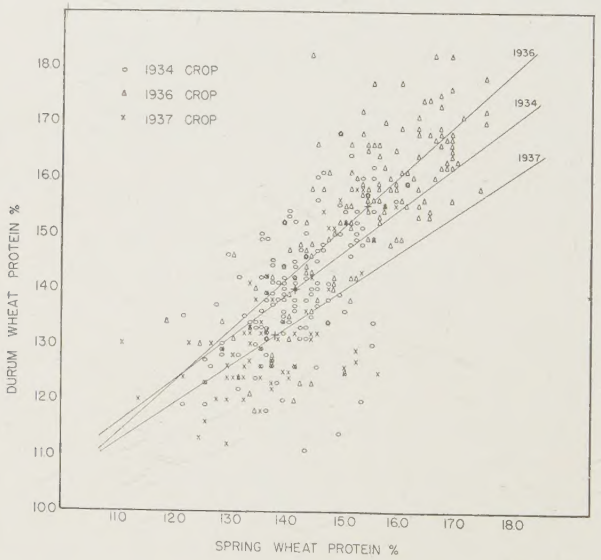

FIGURE 4. Scatter diagram and regressions for mean protein contents of hard red spring and amber durum wheats from corresponding shipping points.

equation for a straight line $y = a + bx$, where y equals barley or durum wheat protein, x equals spring wheat protein, b is the regression coefficient or slope of the line, and a is the y intercept which controls the position of the line. The statistics for the barley-wheat relation show that the regression coefficients for the different years do not differ significantly, i.e., that the chances are somewhat greater than one in twenty that the actual differences between the slopes of the lines, as illustrated in Figure 3,

arise fortuitously rather than because the relation between barley and spring wheat protein changes from year to year. In this connection it should be borne in mind that a study of data for a larger number of years might show that regression coefficients for different years do differ significantly. However, on the basis of the existing data, it may be tentatively assumed that the same regression coefficient, or b, can be used for each annual prediction equation.

On the other hand, the statistics show that the deviations of the means from the average regression are significant. Thus the constant a in the prediction equation will differ from year to year. Consequently it is not justifiable to develop a general equation for the relation between barley and spring wheat which can be applied in any year. The constant a will have to be determined for the crops of each new year and the possibilities of predicting barley protein from spring wheat protein are therefore limited. It seems pertinent to point out also that owing to the looseness of the relation involved, the intra-annual prediction of barley protein from spring wheat protein cannot be particularly accurate.

The statistics for the relation between durum wheat protein and spring wheat protein show that exactly the same situation exists. The regression coefficients for different years do not differ significantly but the positions of the regression lines for the three years do differ significantly. In consequence the possibilities for predicting durum wheat protein from spring wheat protein are also limited to intra-annual studies.

Through the courtesy of the "Crop Testing Plan" there was made available a series of paired samples of O.A.C. 21 barley and Thatcher wheat grown in 1939 on adjacent plots at 285 points in Western Canada. The relation between the protein contents of the barley and wheat samples is illustrated in the scatter diagram, Figure 5. Mean protein contents

TABLE 5.—TEST OF HOMOGENEITY OF YEARLY REGRESSION COEFFICIENTS BY ANALYSIS OF RESIDUAL VARIANCE

Variance due to	Barley and red spring wheat		Amber durum and red spring wheat	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Differences among yearly regression coefficients	2	1.58	2	1.82
Deviation of means from average regression	2	32.94**	2	22.84**
Deviations from individual yearly regressions	504	.62	347	.84

** Significance exceeds the 1% level.

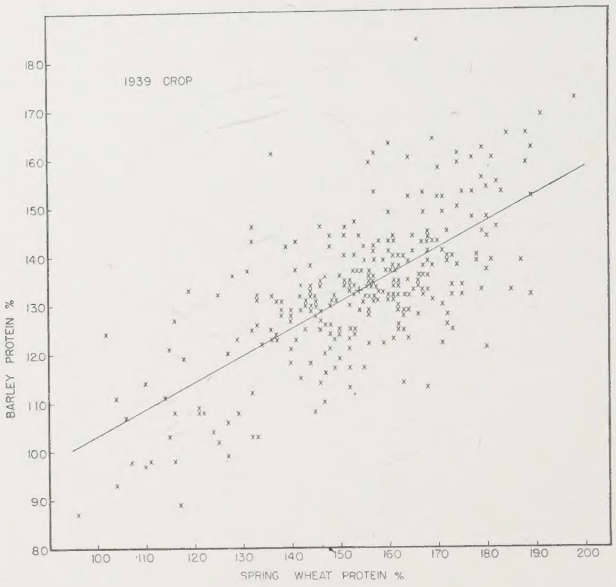

FIGURE 5. Scatter diagram and regression for mean protein contents of paired samples of O.A.C. 21 barley and Thatcher wheat grown on adjacent plots.

were 13.3% for barley and 15.4% for wheat, and protein ranges were 8.7 to 18.4% for barley, and 9.6 to 19.8% for wheat. The coefficient of correlation proved to be 0.70, which is slightly lower than the highest correlation coefficient (0.74) obtained from the protein survey data. There is thus some reason to believe that the fact that the survey data represented several varieties of wheat and barley, and the averages for samples grown in the same district rather than on adjacent plots, had little tendency to reduce the apparent degree of association between barley and wheat protein, as illustrated by the survey data.

The studies, as a whole, indicate that districts which produce hard red spring wheat of high protein content also tend to produce amber durum wheat and barley of high protein content, and that the same situation exists with respect to low protein districts. However, the relations which exist between the protein contents of the three crops are too loose to permit accurate prediction of the protein content of one from the protein content of another. It follows that if detailed information is required on the protein contents of all three crops, it is necessary to make a separate protein survey for each of them. This practice has been followed for several years in the Board of Grain Commissioners' laboratory and appears to be fully justified.

SUMMARY

A study was made of the relation between the protein contents of hard red spring wheat and barley using 510 pairs of values, each pair representing the average protein contents of samples of the two grains collected from the same shipping point. The data were obtained from protein surveys of Western Canadian crops made in the years 1934, 1936 and 1937. A similar study was also made for spring wheat and durum wheat using 353 pairs of values.

The mean protein content of the barley proved to be 1.7% lower, and the value for durum wheat 0.3% lower, than corresponding values for spring wheat. The total intra-annual correlation coefficients were 0.65 for barley and spring wheat and 0.68 for durum and spring wheat. The corresponding coefficients of regression for the protein content of each crop on that of spring wheat were 0.56 for barley and 0.84 for durum wheat.

A further study of the barley-spring wheat relation was made with data for 285 samples of O.A.C. 21 barley and Thatcher wheat grown in 1939 on adjacent plots under "The Crop Testing Plan". The correlation between the protein contents of barley and wheat proved to be 0.70 which is little higher than the value obtained from the less uniform protein survey data.

The relations between the protein contents of each pair of crops are rather loose, and statistical studies indicate that they are not constant from year to year. Although the data show that districts which produce hard red spring wheats of high protein content also tend to produce barley and durum wheat of high protein content, and that the same situation exists with respect to low-protein districts, it is apparent that if detailed information is required on the protein content of the three grains, a separate protein survey must be made for each of them.

A STUDY OF THE TRANSFERENCE OF IMMUNITY TO STEM RUST FROM *TRITICUM DURUM* VAR. IUMILLO TO *T. VULGARE* BY HYBRIDIZATION[1]

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INTRODUCTION

The purpose of the present paper is to report the results of an attempt to transfer to *Triticum vulgare* the immunity to stem rust possessed by *T. durum* variety Iumillo.

Since 1901, when Iumillo wheat was first received from Italy by the United States Department of Agriculture, it has been reported by many workers to be immune or highly resistant to stem rust. It was recognized, however, that this variety contained strains that differed in degree of resistance. Newton and Johnson (9) tested a strain of Iumillo for its reaction in the seedling stage to 22 physiologic races of *Puccinia graminis Tritici* and found it immune to all races. Field tests with artificially-induced epidemics of stem rust involving many physiologic races conducted at the Dominion Rust Research Laboratory over a period of years have shown it to be immune at the mature plant stage also. Since the rust used as inoculum was from collections made throughout Canada, it is probable that the strain of Iumillo under discussion is immune at all stages of growth to all races of stem rust occurring in Canada. Its immunity is not an entirely stable condition; a small proportion of susceptible plants sometimes occurs. It was the above mentioned strain of Iumillo that was used in the studies here reported.

A number of wheat breeders have made crosses between Iumillo and varieties of *T. vulgare* and have produced Vulgare-like wheats possessing a high degree of rust resistance, but, as far as the writers are able to determine, none of these wheats has been shown to possess the full immunity of Iumillo. Probably the most successful attempt was that of the University of Minnesota in co-operation with the United States Department of Agriculture. Through this work, the results of which have been reported by Hayes and his co-workers (3, 4, 5), Marquillo wheat was selected from the cross Marquis × Iumillo and Thatcher wheat from the double cross (Marquis × Iumillo) × (Marquis × Kanred). Both Marquillo and Thatcher are, as a rule, highly resistant to stem rust under field conditions in North America, but they sometimes show considerable infection. In the seedling stage both varieties are immune to some races of stem rust but susceptible to others. Marquillo must have derived its complex of factors for rust resistance chiefly from Iumillo since its other parent, Marquis, is susceptible to many races of rust. Thatcher, on the other hand, derives its resistance from both Iumillo and Kanred.

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For some years the varieties chiefly used as a source of rust resistance in the wheat breeding work at the Dominion Rust Research Laboratory were Hope and H-44. They are resistant at the mature plant stage to all physiologic races of stem rust but susceptible at the seedling stage to many races. The mature plant resistance of these varieties is inherited on either a monofactorial or a bifactorial basis in most crosses with susceptible wheats, so that large numbers of resistant derivatives are easily obtained; but many of these tend to be susceptible to heat and drought damage and to diseases causing a blackening of the stems and glumes. It appeared desirable to investigate other sources of rust resistance, and Iumillo, due to its immunity to stem rust at all stages of growth, was chosen for study.

In reporting work of this kind it seems desirable that some definite usage be established for terms which include a complex of varieties all belonging to one botanical species or group of species. Throughout this paper the authors propose to use the common term "Emmer" to refer to all species of wheat with 28 chromosomes, including *T. dicoccoides* Körn., *T. dicoccum* Schülb., *T. durum* Desf., *T. Persicum* Vav., *T. orientale* Perc., *T. pyramidale* Perc., *T. Polonicum* L., and *T. turgidum* L. as well as *T. Timopheevi* Zhuk.; the term "Durum" to refer particularly to all varieties of the botanical species *T. durum* Desf.; and the term "Vulgare" to refer to all varieties of the 42-chromosome species *T. vulgare* Host.

With further reference to terminology, the term "immunity" is here used to mean complete resistance.

MATERIALS AND THEIR ORIGIN

The materials on which the present studies were made consisted of 50 selected hybrid lines of wheat derived from a breeding project begun at the Dominion Rust Research Laboratory in 1930 by Dr. K. W. Neatby, for the purpose of transferring to *Triticum vulgare* the immunity to stem rust possessed by Iumillo. This project was under his direction until the spring of 1935, after which time it was conducted by the senior author. In order to explain the nature of the 50 lines in question, a brief account of their origin is presented below.

Six crosses were made, and the parent varieties used, with the exception of Iumillo, were varieties of *Triticum vulgare* and all of the spring habit. The crosses made by Dr. Neatby and the approximate number of F_2 plants grown were as follows:

Cross	Year of Cross	Number of F_2 plants
Marquis × Iumillo	1930	2,600
Iumillo × Hope	1931	2,400
Iumillo × R.L. 729	1931	6,000
Iumillo × Garnet	1931	1,120
Marquillo × Iumillo	1932	4,900
Ceres × Iumillo	1932	1,100

Marquis C.A.N.[1] 1396, Garnet C.A.N. 1316, and Ceres C.A.N. 1263 are susceptible to many physiologic races of stem rust. Hope C.A.N. 1339 (derived from a Marquis × Yaroslav Emmer cross) and Marquillo C.A.N. 1408 have been discussed above. R.L. 729 (derived from a Pentad Durum × Marquis cross) has mature plant resistance similar to that of Hope.

Some of the F_1 plants were grown in the greenhouse and the remainder were grown in the field and dusted with sulphur to prevent rust damage. The F_2 and succeeding generations were grown in the field under artificially-induced epidemics of stem rust involving approximately 30 physiologic races each year. Attacks from other physiologic races occurred naturally. In F_2, plants having a considerable number of Vulgare characteristics were selected, and from the seed of each of these an F_3 plot was grown. In F_3, Vulgare-like plants showing resistance to stem rust were selected (often several plants from a single plot) and from the seed of each of these an F_4 plot was grown. This process was continued up to the fall of 1936 and each year the selection for rust resistance and Vulgare morphology was made more rigorous. In 1936, instead of single plants, entire plots apparently immune to stem rust were harvested.

In addition to the field selection described above, some selection was done in the greenhouse during the winter of 1934–35 among the progeny of plants from the various crosses grown in 1934. Tests were made for seedling reaction to Physiologic Race 21 (a race that attacks a wide range of Vulgare wheats) in order to eliminate susceptible material. Plants immune or highly resistant to this race were kept for further study. On the whole, however, the selection for rust resistance was based on the reaction of plants approaching maturity in the field.

Fifty plots were found to be free from rust in 1936 and these represented all crosses except Ceres × Iumillo and Iumillo × Garnet in which no rust-free plots were found. These 50 plots and their progeny are referred to as "lines" in this paper. Table I shows the origin of the 50 lines. It will be seen that they are derived from 14 F_2 plants.

[1] Canadian Accession Number.

TABLE 1.—THE ORIGIN OF 50 RUST-RESISTANT LINES OF WHEAT

Line number	Cross	Generation in 1936	Origin of Lines (F_2 Plant Number)
1 to 6 inclusive	Marquis × Iumillo	F_6	C-30-3
7 to 9 inclusive	Marquis × Iumillo	F_6	C-30-41
10 to 21 inclusive	Iumillo × Hope	F_6	C-31-20
22 and 23	Iumillo × Hope	F_5	C-31-15
24 and 25	Iumillo × Hope	F_5	C-31-20
26	Iumillo × Hope	F_5	C-31-22
27 and 28	Iumillo × R.L. 729	F_6	C-31-10
29 and 30	Iumillo × R.L. 729	F_6	C-31-31
31 and 32	Iumillo × R.L. 729	F_5	C-31-5
33 to 35 inclusive	Iumillo × R.L. 729	F_5	C-31-10
36 and 37	Iumillo × R.L. 729	F_5	C-31-18
38 and 39	Marquillo × Iumillo	F_4	C-32-3
40	Marquillo × Iumillo	F_4	C-32-8
41 to 43 inclusive	Marquillo × Iumillo	F_4	C-32-11
44 and 45	Marquillo × Iumillo	F_4	C-32-18
46 to 50 inclusive	Marquillo × Iumillo	F_4	C-32-27

It has been stated that lines approaching the Vulgare type were selected. Exceptions were made in the case of two lines (Nos. 38 and 39) which possessed some Vulgare characteristics but on the whole inclined more to the Durum type. Due to their apparent immunity to stem rust it seemed desirable to test them in comparison with the 48 Vulgare-like lines. Thus the material under study consisted of 48 Vulgare-like lines and 2 Durum-like lines possessing an appreciable number of Vulgare morphological characters.

EXPERIMENTAL METHODS AND RESULTS

Studies were made of the fifty selected lines in order to determine to what extent the aim of securing Vulgare wheats with the rust immunity of Iumillo had been achieved. These studies included tests of reactions to stem rust in both the mature and seedling stages, chromosome counts and other cytological studies, and morphological studies designed to determine the degree of "Vulgare-ness" of the various lines. In addition, certain of the lines were crossed with Marquis and with Iumillo to obtain an indication of their value as a source of stem rust resistance in further breeding work. The methods and results of the various studies are given below.

1. Field Tests for Rust Reaction

Plots containing 40 to 60 plants of each line, and similar plots of the parent varieties, were grown in the field under an artificially-induced epidemic of stem rust involving approximately 30 physiologic races. When the plants had reached maturity, an estimate was made of the percentage of rust in each plot according to the "Scale for Estimating Rust" adopted by the Office of Cereal Crops and Diseases of the United States Department of Agriculture (2).

The stem rust readings given in Table 2 show that the 50 lines, selected for their immunity in 1936, were for the most part, free from stem rust in 1937 and 1938. In a few lines a small proportion of the plants was rusted. Some of these were obviously due to natural crossing of the lines with susceptible wheats. Possibly some were due to cytological irregularities. Marquis was heavily rusted in all three years.

While the present studies were chiefly concerned with stem rust reactions, the reactions of the 50 hybrid lines of wheat to the leaf rust organism, *Puccinia triticina* in the field were determined by Dr. Margaret Newton and Mr. B. Peturson. With their kind permission the average readings are included in Table 2. The determinations were made after the plants had headed, so that the percentages given represent the mature plant reactions. It will be seen that some lines were resistant and that others were susceptible or exhibited an intermediate reaction. A number of lines were not breeding true for leaf rust reaction.

2. Greenhouse Tests of Seedling Reactions to Stem Rust

Seedlings of the 50 selected lines and of the 5 parent varieties were tested in the greenhouse during the winter of 1936–1937 for their reaction to 9 physiologic races of stem rust. For a test 15 seeds of each line or

TABLE 2.—PERCENTAGE STEM RUST AND LEAF RUST ON MATURE PLANTS IN THE FIELD, "VULGARE-NESS" SCORES, AND CHROMOSOME NUMBERS IN 50 RUST-RESISTANT LINES OF WHEAT AND PARENT VARIETIES

Lines or varieties tested	Stem rust,* %			Leaf rust %	Score for "Vulgare-ness"			No. of plants studied cyto-logically	Chromosome numbers	
	1936	1937	1938	1938	Mean	Low	High	1937	Usual	Also found
Marquis × Iumillo										
1	0	0	0	67	13.7	9.0	16.2	5	41	42
2	0	0	(0)	70	13.8	11.2	16.0	6	42	41
3	0	0	0	65	14.1	12.8	16.0	5	42	41
4	0	0	0	65	13.8	12.8	15.0	5	42	40, 43
5	0	0	(0)	72	12.9	8.2	14.0	6	42	41
6	0	0	0	67	14.0	12.5	15.8	5	41	42
7	0	0	0	5	12.1	7.5	14.8	5	41	42
8	0	0	0	3	12.8	10.2	14.5	5	42	41
9	0	0	0	3	13.1	11.5	14.5	8	42	41
Iumillo × Hope										
10	0	0	0	10	16.2	13.8	17.8	7	41	39, 40, 42, 43
11	0	0	0	Trace	16.2	14.0	18.0	9	42	
12	0	0	0	5	15.7	11.8	17.5	5	42	40. 41
13	0	0	0	5–55	15.4	13.8	17.2	5	39	40, 41, 42
14	0	0	0	22	16.8	14.5	17.5	8	41	42
15	0	0	0	Tr–15	14.7	13.2	16.5	7	40	39
16	0	0	0	Trace	12.0	9.2	13.8	4	40	
17	0	0	0	20	15.4	9.0	18.0	7	40	39, 42
18	0	0	0	22	16.2	13.8	18.0	9	41	42
19	0	0	0	15	15.1	8.8	17.8	7	40	39, 41, 42
20	0	0	0	12	15.8	11.5	18.0	6	40	41
21	0	0	0	5–35	15.0	8.8	18.0	8	40	39, 41, 42
22	0	0	0	Trace	13.8	10.0	17.2	5	40	39
23	0	0	0	Trace	13.8	9.5	16.0	5	40	39
24	0	0	0	Trace	15.4	13.2	17.2	7	40	39, 41
25	0	0	0	Tr–40	14.4	8.2	17.0	6	41	40, 42
26	0	(0)	0	5–35	14.4	10.2	17.5	5	41	38, 39, 40
Iumillo × R..L. 729										
27	0	0	(0)	40	16.5	14.5	17.2	5	42	41
28	0	0	0	40	15.5	13.2	17.5	7	42	
29	0	0	0	67	15.4	13.0	17.8	7	42	43
30	0	0	0	67	15.4	12.0	17.0	9	42	
31	0	0	(0)	Trace	14.3	12.2	16.8	5	41	42, 43
32	0	0	0	Tr–50	15.2	11.0	17.2	5	42	41
33	0	(0)	0	40–60	15.5	13.8	17.0	9	41	40, 42
34	0	0	0	37	14.8	11.8	17.2	7	41	43
35	0	0	0	15	15.7	14.2	17.5	8	42	
36	0	0	0	Trace	13.0	7.8	16.8	6	41	42
37	0	0	0	Trace	14.3	11.8	16.2	6	42	41

* In lines marked thus, (0), the plants were typically free from rust but a small proportion of rusted plants was present.

TABLE 2.—PERCENTAGE STEM RUST AND LEAF RUST ON MATURE PLANTS IN THE FIELD, "VULGARE-NESS" SCORES, AND CHROMOSOME NUMBERS IN 50 RUST-RESISTANT LINES OF WHEAT AND PARENT VARIETIES—*Concluded*

Lines or varieties tested	Stem rust,* %			Leaf rust %	Score for "Vulgare-ness"			No. of plants studied cyto-logically	Chromosome numbers	
	1936	1937	1938	1938	Mean	Low	High	1937	Usual	Also found
Marquillo × Iumillo										
38	0	0	0	5	6.5	4.5	7.5	6	28	
39	0	0	0	Trace	6.3	5.0	7.2	6	28	
40	0	(0)	0	5–20	15.7	12.8	17.5	7	40	41
41	0	0	0	Trace	15.6	11.0	17.5	10	41	39, 40
42	0	(0)	0	5	15.3	13.0	17.8	7	39	38
43	0	0	0	17	15.5	11.2	17.8	7	39	38, 40, 41
44	0	0	0	25	16.3	14.0	17.8	9	41	39, 42
45	0	0	0	27	15.5	12.0	17.5	8	42	41
46	0	0	0	72	16.0	8.0	18.0	7	42	41
47	0	(0)	(0)	5–70	15.9	12.5	17.8	9	42	41
48	0	(0)	(0)	72	16.1	14.2	17.8	7	42	41
49	0	(0)	0	5–20	16.2	10.8	17.5	9	42	41
50	0	(0)	(0)	Tr–15	15.4	12.2	17.5	10	42	
Parental varieties										
Iumillo	0	0	0	Trace	0.0			4	28	
Marquis	75	85	80	55	18.0			4	42	
Hope	Trace	Trace	0	Trace	18.0			5	42	
R.L. 729	Trace	Trace	0	Trace	18.0			4	42	
Marquillo	5	10	Trace	65	18.0			6	42	

* In lines marked thus, (0), the plants were typically free from rust but a small proportion of rusted plants was present.

variety were shown in each of 2 pots and the resulting seedlings inoculated with a pure culture of stem rust, the usual technique (9) being followed. Each test was repeated so that the final average reading was based on approximately 60 seedlings grown in 4 pots. Individual seedlings were classified into 6 categories and, for purposes of computation, these were assigned numerical values as follows:

Reaction	Numerical value	Reaction	Numerical value
Immune	0	Intermediate	3
Resistant	1	Moderately susceptible	4
Moderately resistant	2	Susceptible	5

The average seedling score within each line or variety was determined for each test and for duplicate tests combined. An indeterminate reaction or so-called "X-reaction" occurred frequently in this material. In these cases rust pustules of types belonging to both resistant and susceptible

classes appeared on the same leaf. The procedure followed was to estimate an average reading for such a leaf. If either the resistant or susceptible type of pustule predominated, this would be reflected in the estimated average.

Table 3 shows the mean seedling reactions of the hybrid lines and their parents to 9 physiologic races of stem rust. The seed used in these tests, both for the hybrid lines and the parent varieties, came from small plots grown side by side in the field. There had undoubtedly been some natural crossing but, as the natural hybrids could not very well be distinguished in the seedling stage, the reactions of all seedlings were recorded and used in calculating the means. In some cases a line appeared typically immune to a certain race of rust but contained a small proportion of susceptible plants so that its mean reaction was higher than zero. This happened in Iumillo itself. The hybrid lines, being less stable cytologically than Iumillo, might be expected to be more subject to natural crossing and to contain more off-types than that variety. The cytological instability itself might be responsible for some susceptible off-types. Under these conditions it is difficult to classify the lines on a strict basis of immunity of non-immunity, but it is probably safe to assume that those with a score of 0.5 or less are typically immune. Intermediate scores are due, in some cases, to an intermediate reaction and in others to a number of different reactions occurring in the same line. The main purpose of the table, however, is to show what lines are immune or highly resistant in the seedling stage to stem rust.

It appears from the data in Table 3 that the 50 lines were preponderantly resistant or immune to stem rust. Of the 441 average readings obtained, 361 were below 3.0, indicating resistance. Of the 48 lines for which complete data were obtained, 17 (derived from five F_2 plants) were immune or resistant to all 9 physiologic races and no line was susceptible to all. In the remaining 31 lines a susceptible or intermediate mean reaction to one or more physiologic races was obtained. No line had the full immunity of Iumillo.

In the field tests reported above most of the physiologic races shown in Table 3, along with others, were used as inoculum. Since the lines were immune to all these races in the field they appear to possess mature plant immunity to them in addition to the seedling immunity or resistance to certain races.

3. Chromosome Counts and Other Cytological Studies

In 1937, 25 seeds of each of the 50 selected lines and of the 5 parent varieties were sown in plots at the Cereal Division, Central Experimental Farm, Ottawa. Permanent iron-aceto-carmine smear slides were made of the anthers at the appropriate stage for observation of meiotic chromosomes. A total of 359 plants was examined cytologically, this total representing from 4 to 10 plants in each of the 50 lines and 5 parents.

The chromosome numbers found are given in Table 2. A summary of the results by crosses and generations appears in Table 4. The percentage of 42-chromosome plants ranged from 3.5 in F_6 Iumillo × Hope to 85.7 in F_7 Iumillo × R.L. 729. Of the 336 plants examined in the 50 lines

TABLE 3.—THE MEAN SEEDLING REACTIONS OF 50 HYBRID LINES OF WHEAT AND OF THE PARENTAL VARIETIES TO NINE PHYSIOLOGIC RACES OF *Puccinia graminis Tritici* ERICKSS. AND HENN.*

Lines or varieties tested	Physiologic Races								
	0	15	21	32	34A	36	38	39	49
1	0.2	3.2	0.3	1.5	1.6	3.1	3.1	4.0	0.1
2	0.0	2.9	0.0	2.0	1.2	3.3	1.9	3.8	0.0
3	0.0	3.5	0.3	1.9	1.3	3.6	2.7	3.8	0.1
4	0.0	3.6	0.2	3.0	2.3	4.3	1.7	2.2	0.0
5	0.1	2.9	0.0	1.1	1.2	1.9	0.9	0.1	0.0
6	0.1	3.7	0.7	2.8	3.0	4.2	0.9	0.1	0.1
7	2.1	0.2	0.4	0.1	0.2	2.1	0.5	1.0	0.8
8	2.7	1.3	1.1	0.6	0.3	2.7	2.5	2.3	0.8
9	2.5	2.0	1.6	0.4	0.3	3.4	3.0	3.5	2.2
10	1.2	1.5	1.4	0.4	1.9	0.0	0.7	1.3	0.1
11	2.6	3.8	3.3	2.8	4.0	0.0	2.2	2.4	0.3
12	0.9	1.0	1.1	0.7	1.1	1.7	1.7	1.6	2.3
13	0.6	0.7	1.0	0.2	0.6	1.6	0.6	0.5	1.1
14	1.1	0.9	1.5	0.6	1.0	0.0	1.6	1.7	0.1
15	0.1	0.5	1.4	0.3	1.3	1.2	0.2	0.5	0.7
16	0.6	0.4	1.4	0.4	1.8	0.4	0.6	0.9	0.3
17	1.5	1.0	2.0	0.4	1.9	0.0	1.5	1.4	0.1
18	0.5	0.4	1.4	0.1	0.7	1.8	1.6	1.5	1.6
19	1.2	0.9	1.5	0.3	0.7	2.0	1.3	0.6	2.4
20	0.4	0.6	1.2	0.4	0.6	2.7	1.5	0.9	1.0
21	0.4	0.8	1.9	0.5	0.8	2.2	1.6	0.9	0.8
22	3.0	2.3	3.0	1.9	3.7	4.2	2.3	1.1	1.9
23	3.0	3.6	3.5	2.5	4.5	4.7	2.0	1.1	3.6
24	2.7	3.9	4.4	3.1	4.8	4.0	3.1	2.4	4.1
25	1.0	2.2	3.0	1.8	3.2	1.8	1.8	1.1	1.8
26	1.8	0.4	2.2	0.8	0.4	3.0	2.8	2.9	2.4
27	1.7	1.8	0.7	0.3	0.2	2.4	1.7	1.9	2.6
28	1.2	1.3	1.1	1.0	0.5	2.6	2.0	1.0	3.3
29	2.9	2.1	2.3	1.6	0.7	1.6	2.4	1.5	2.8
30	3.6	2.5	2.5	2.1	2.2	0.1	2.2	2.2	1.0
31	2.5	3.7	3.6	3.5	3.9	4.3	1.4	0.8	3.6
32	2.2	1.7	2.3	0.7	0.3	4.0	0.5	0.5	2.4
33	3.0	1.8	2.2	2.2	1.8	3.7	2.1	2.1	2.8
34	3.0	2.3	2.4	1.4	0.8	2.8	2.0	2.1	2.8
35	4.4	4.4	4.4	3.9	4.2	2.6	3.5	3.5	3.7
36	3.0	2.7	3.2	2.4	3.3	2.8	0.9	0.8	2.3
37	2.2	3.3	3.4	3.5	3.5	4.4	1.2	1.2	2.7
38	-	-	-	-	0.8	0.9	0.8	-	0.0
39	-	-	-	-	1.0	1.7	1.1	2.2	0.2
40	0.8	1.0	0.4	1.2	0.6	0.9	1.8	3.0	1.1
41	0.6	0.3	0.7	0.2	0.4	0.1	0.7	3.5	1.2
42	0.5	1.0	0.5	0.5	0.3	1.5	0.6	0.0	1.1
43	1.3	0.6	0.6	0.4	0.2	0.5	0.4	1.0	0.9
44	2.5	2.8	1.4	0.5	0.9	3.1	2.8	3.5	3.7
45	2.9	2.0	1.7	0.6	0.8	3.5	3.5	3.5	3.4
46	2.8	2.0	0.7	0.6	0.6	3.3	3.4	3.4	3.0
47	3.5	2.8	1.9	1.2	1.1	3.3	2.6	2.4	3.3
48	3.1	1.8	0.7	0.2	0.4	3.2	2.8	2.4	3.0
49	2.5	2.0	0.8	0.4	0.4	1.7	3.4	2.8	2.5
50	3.0	2.8	1.8	0.4	0.7	2.6	2.8	3.0	3.0
Iumillo	0.0	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.0
Marquis	4.8	5.0	5.0	4.9	4.7	5.0	2.0	2.2	3.5
Hope	4.4	5.0	5.0	5.0	4.9	0.2	2.1	2.5	1.3
R.L. 729	4.8	4.6	5.0	4.9	5.0	0.8	2.6	2.8	1.6
Marquillo	2.9	3.8	3.1	2.8	3.6	4.0	2.3	2.5	4.1

* The range of scores is from 0 (complete immunity) to 5 (complete susceptibility).

140 (41.6%) had 42 chromosomes. All plants examined in 5 lines (Nos. 11, 28, 30, 35 and 50) had 42 chromosomes, and all plants examined in 2 lines (Nos. 38 and 39) had 28 chromosomes. In 29 lines some, but not all, of the plants had 42 chromosomes. In the remaining 14 lines no 42-chromosome plant was found. These results are only an indication of the chromosome numbers occurring in the different lines. By examining a larger number of plants in each line, chromosome numbers other than those reported might be found. A summary of this part of the project has been published elsewhere (7).

Table 4 shows that there was a higher proportion of 42-chromosome plants in F_7 than in F_6 lines of the 2 crosses Iumillo × Hope and Iumillo × R.L. 729, and this was probably due to selection for the Vulgare type of head. The reason for the better results in the latter than in the former cross may be that there was more material in F_2 from which to select Vulgare types.

TABLE 4.—CHROMOSOME NUMBERS IN VULGARE-LIKE DERIVATIVES FROM VULGARE-IUMILLO CROSSES

Material	No. of lines	Number of plants with the chromosome number							Total no. of plants	Per cent plants with 42 chrom.
		28	38	39	40	41	42	43		
Marquillo × Iumillo F_5	13	12	2	14	8	19	47	—	102	46.0
Iumillo × Hope F_6	5	—	1	4	14	8	1	—	28	3.5
Iumillo × Hope F_7	12	—	—	9	26	25	21	1	82	25.6
Marquis × Iumillo F_7	9	—	—	—	1	22	26	1	50	52.0
Iumillo × R. L. 729 F_6	7	—	—	—	1	22	21	2	46	45.6
Iumillo × R.L. 729 F_7	4	—	—	—	—	2	24	2	28	85.7
Totals	50	12	3	27	50	98	140	6	326	41.6

A detailed account of the chromosome behaviour of the 50 lines will be published separately. It may be mentioned here, however, that the aberrant chromosome behaviour can be ascribed to three phenomena: first, aneuploidy (many plants had not completely reverted to the Vulgare condition of 42 chromosomes, that is, they contained one or more unpaired chromosomes which frequently failed to be incorporated in the pollen nuclei); second, specific irregularities such as translocations, deletions and duplications of chromosome segments (these were found in 15% of the plants examined at the first meiotic division, and inversions were found in 50% of the 175 plants studied at the young pollen tetrad stage); and third, gene-cytoplasm unbalance in some of the hybrid derivatives (6).

Approximately 50% of all plants examined exhibited specific irregularities due to rearrangements of chromosome segments. In fact, 57 of the 140 plants with 42 chromosomes exhibited such rearrangements. At least 12 plants had more than one type of abnormality.

All plants examined in lines 11, 28, 30, 35 and 50 had 42 chromosomes, but none of these lines was free from specific irregularities. Line 11 contained 2 plants (out of 9) with an inversion and 1 plant with an association

of 4 chromosomes to form a chain. Line 28 contained 3 plants (out of 7) with 1 or 2 inversions. Line 30 contained 4 plants (out of 9) with an inversion and 1 plant with an association of 4 chromosomes to form a ring. Lines 35 and 50 contained 4 and 2 plants (out of 8 and 10) respectively, with inversions.

Out of the 140 plants with 42 chromosomes, 81 appeared to be relatively stable cytologically. Seeds from these plants were sent to Winnipeg as material for further breeding work.

4. Morphological Studies

The plant characters and methods used in the morphological studies were essentially those used by Thompson *et al.* (10). The actual characters used, 19 in all, were as follows: stem diameter, stem cavity (present or absent), collar (closed or open), density of spike, form of spike, beards (present or absent), shape of glume, cross-section of glume (V- or U-shaped), beak of glume (acuminate or acute), shoulder of glume (narrow or wide, oblique or square), keel (prominence), keel teeth (shape), rachis segment (slender or stout), upper rachis hairs (arrangement), marginal rachis hairs (arrangement), seed shape, seed cross-section, embryo end of seed (shape), and seed hairs (number and length).

Of the 19 characters named above it was possible to use only 18 in each cross. The glume beak character of Hope and R.L. 729 resembles very closely that of Iumillo. The same character in Marquillo varies from a Marquis type to a near-Iumillo type in different plants. In crosses involving these three Vulgare wheats, this character was not included in the counts. In the cross Marquis × Iumillo the glume shoulder character was omitted from the counts because, due to the variability of expression, it was impossible to assign a definite numerical value to the condition of the glume shoulder in many of the derivatives.

The condition of each character (i.e. Vulgare or Durum) was determined for each plant, an average of 16 plants in each line being studied. A numerical estimate of the "Vulgare-ness" of the plants was obtained by assigning the following values to the condition of each character found in each plant:

Condition of character	Numerical value	Condition of character	Numerical value
Durum	0.00	Nearly Vulgare	0.75
Nearly Durum	0.25	Vulgare	1.00
Intermediate	0.50		

The total score for each plant was obtained (the maximum possible score in each case being 18) and this total was considered the "Vulgare-ness" score for the plant in question. The average "Vulgare-ness" score for each line was also determined.

The scores for "Vulgare-ness" are shown in Table 2. A full score of 18 indicates that the plant or line in question resembled the Vulgare parent in all 18 characters. A score of zero means resemblance to the Durum wheat, Iumillo, in all characters. Intermediate scores indicate to what extent the lines resembled one or the other parent.

It should be noted that the scores for "Vulgare-ness" given in Table 2 are comparable within but not between crosses since in each cross the Vulgare parent was used as a standard with a full score of 18, although the 4 Vulgare parents differed with respect to some characters. If we take Marquis as an arbitrary standard of "Vulgare-ness", the varieties Hope, R.L. 729, and Marquillo are found to have some morphological characters tending toward the Emmer type. On this basis the average scores for the parent varieties, based on all 19 characters, were found to be as follows:

Variety	Score	Variety	Score
Marquis	19.0	Hope	13.6
Marquillo	17.6	Iumillo	0.0
R.L. 729	15.0		

However, in scoring plants from the cross Iumillo × Hope, the condition of each character as found in Hope was taken as the Vulgare standard and given a score of 1.00. Similarly in the crosses Marquillo × Iumillo and Iumillo × R.L. 729 the condition of the characters in Marquillo and R.L. 729 respectively was taken to be the Vulgare condition. The morphological scores for plants in the three crosses mentioned were, therefore, somewhat higher than they would have been had Marquis been used as a standard throughout the studies.

In Table 5 the average morphological scores of plants with different chromosome numbers are tabulated. These are given by cross and generation. The data show that plants with 38 to 43 chromosomes approached the Vulgare phenotype much more closely than those with 28 chromosomes. It is apparent, however, that the genes for the Vulgare condition of characters were not confined to the so-called C set of chromosomes.

For comparison, 39-chromosome plants with 19 pairs and 1 single chromosome and those with 18 pairs and 3 single chromosomes are listed separately. The 40-chromosome plants with 20 pairs and those with 19 pairs and 2 single chromosomes are similarly treated. In each of the 39- and 40-chromosome groups the plants listed in the second column probably have one more "C" chromosome than those in the first. In comparing the morphological scores in these columns, 6 comparisons are available. There is an average difference of 2.0 in favour of the second column but this difference is not statistically significant.

TABLE 5.—AVERAGE VULGARE SCORE FOR PLANTS WITH DIFFERENT CHROMOSOME NUMBERS

Cross and generation	Chromosome numbers								
	28	38	39		40		41	42	43
			1_I	3_I	0_I	2_I			
Marquillo × Iumillo F_5	7.0	12.9	11.0	16.4	15.5	15.4	15.7	16.2	—
Iumillo × Hope F_6	—	15.0	15.5	12.5	10.7	14.6	15.6	15.5	—
Iumillo × Hope F_7	—	—	14.2	16.5	12.5	16.1	16.4	16.5	16.8
Marquis × Iumillo F_7	—	—	—	—	—	14.8	12.2	13.5	14.3
Iumillo × R.L. 729 F_6	—	—	—	—	—	15.8	14.8	15.5	15.7
Iumillo × R.L. 729 F_7	—	—	—	—	—	—	16.8	15.5	15.7

5. Crosses Involving the Hybrid Lines

In order to get an indication of the possible value of the hybrid derivatives as a source of rust resistance in crosses, Line 9 (see Table I) was crossed with Marquis and the F_2 grown in the field in 1939 under an artificially-induced stem rust epidemic involving at least 30 physiologic races. The cross Line No. 1 × Iumillo was also made and treated in the same way in an attempt to develop a hybrid line approaching Iumillo still more closely in degree of rust resistance.

From the cross Line 9 × Marquis, 485 F_2 plants (all of the Vulgare type) were obtained and 11 of these were free from rust under conditions where the degree of infection of Marquis was moderately high. The inheritance of rust resistance in this case is obviously not simple, but yet it does not appear to be too complex to be of use in a breeding program. From the cross Line 1 × Iumillo, 75 F_2 plants were developed. In head type they varied from near-Vulgare to near-Durum and all plants were free from stem rust. These will be further studied but it would appear from the present results that Line 1 and Iumillo possess in common, one or more factor pairs capable of causing mature plant resistance to stem rust under field conditions.

DISCUSSION

1. Rust Reactions

Although the present study deals with the characteristics of selected hybrid lines, and is not a study of the mode of inheritance of rust immunity based on random hybrid plants or lines, some of the results, considered in the light of previous studies, are of interest from the standpoint of genetics.

It would appear from the results of the field tests that the 50 hybrid lines of wheat under study were, like Iumillo, immune in the mature plant stage to all physiologic races present in the field under the conditions of trials conducted over a period of three years at Winnipeg. The greenhouse tests, on the other hand, show clearly that although all 50 lines had inherited some stem rust resistance from Iumillo, none had the full immunity to all races of rust of that variety in the seedling stage.

The 9 lines of Marquis × Iumillo and the 13 lines of Marquillo × Iumillo presumably derived their stem rust resistance or immunity from Iumillo, either directly or through Marquillo (a derivative of Marquis × Iumillo). The lines of Iumillo × Hope and Iumillo × R.L. 729, however, may have genes for the mature plant resistance of Hope or R.L. 729 with additional factors for resistance from Iumillo. Iumillo must have contributed some factors for resistance to these lines since all of them have more resistance than either Hope or R.L. 729.

In the various studies that have been made of crosses between Iumillo and susceptible varieties of *T. vulgare* the great variability of F_2 plants as regards chromosome number and cytological behaviour has made it impossible to determine the exact factorial basis of stem rust resistance and susceptibility. In the F_2 generation of such crosses various degrees of rust resistance have been found and the proportion of immune plants of all morphological types has been small, indicating a complex type of inheritance. Further evidence of the complexity of inheritance is to be found in

the work of Neatby and Goulden (8) on Marquillo, a Marquis × Iumillo derivative possessing part of the Iumillo resistance. In the cross Garnet × Marquillo only one F_2 plant in a sample of over 5,000 possessed resistance equal to that of Marquillo; and in the cross Marquillo × Reward only one F_2 plant of a total of 1,598 was as resistant as Marquillo. Other crosses with Marquillo yielded a low proportion of resistant plants and all the evidence indicated that Marquillo differed from the susceptible varieties in question by several or many genes governing rust resistance. This would suggest that the inheritance of resistance in Iumillo × *T. vulgare* crosses is also complex.

In the present study, the small number of immune lines with Vulgare characters obtained from the crosses does not in itself prove that the inheritance is complex involving many genic differences: a relatively simple genic condition could give few such plants if there were linkage (either in the strict sense or resulting from chromosome incompatibility) between immunity and the Durum type. Evidence of such linkage has been noted but in various crosses between Iumillo and susceptible Vulgare wheats the proportion of immune plants of all types in F_2 is low. The separation of two types of resistance (seedling and mature plant resistance) in the hybrids would appear to exclude the possibility of the immunity of Iumillo being controlled by a single pair of allelomorphs. Furthermore, the various seedling reactions to the different physiologic races of rust found in the selected lines as contrasted with the immunity of Iumillo to all races indicates a quite complex inheritance of seedling immunity, but this does not rule out the possibility of a simpler inheritance of mature plant immunity. The results of the cross Line 9 × Marquis indicate that these varieties differ by more than one pair of genes governing mature plant resistance.

Since the chromosomes of Iumillo belong to the so-called A and B sets, the genes of that variety governing stem rust immunity must be in the A or B set or both. The Vulgare wheats possess the so-called C set in addition to the A and B sets and it would appear possible that the difficulty of obtaining fully immune Vulgare types is due to genes in the C set inhibiting or reducing the effect of the genes for immunity. If this be the case it should be possible to obtain immune Vulgare wheats through translocations of parts of chromosomes involving the A or B set on the one hand and the C set on the other. That such translocations must occur through crossing-over between the sets is shown by the occurrence of multivalents in the F_1 (unpublished data of the junior author). Whatever the fundamental basis of the difficulty may be, it seems to have been largely overcome in Line 1 since, in crossing it with Iumillo, all F_2 plants obtained had mature plant immunity.

A complex inheritance in *T. vulgare* × *T. durum* crosses does not preclude the possibility of a simpler inheritance in intra-Vulgare crosses once the desired character has been transferred from *T. durum* to *T. vulgare*. In some of the present cases such a simplification seems to have been achieved. The cross Line 9 × Marquis yielded 11 rust immune and a number of partially resistant plants in an F_2 population of 485 plants, whereas in a cross such as Marquis × Iumillo the proportion of immune plants is considerably smaller. Line 9 and similar lines have, therefore, some promise as breeding material.

2. Cytological Behaviour

The rearrangements of chromosome segments found in many of the plants examined may have been present in the original F_1 or they may have arisen in later generations. It has been stated (10) "There is no evidence of such translocations [between chromosomes of the primary (A and B) and secondary (C) sets of chromosomes] in the numerous reports of the behaviour of the chromosomes at meiosis in hybrids". Unpublished data of the junior author, however, show definitely that the F_1 of many Vulgare-Durum crosses is heterozygous with respect to the arrangement of segments of one or more chromosomes. There is little doubt that further shifts of genes occur between chromosomes of the different sets in later generations as well.

3. Morphological Studies

Arnason (1) has reported the transference of a number of Durum characters to 42-chromosome plants by crossing. In general, the morphological studies herein reported corroborate his results. His report was based on 42-chromosome plants. The present study deals with segregates having 28 to 43 chromosomes. In this study it was difficult to eliminate all Durum morphological characters from 42-chromosome plants while retaining a high degree of the Durum resistance to stem rust. This may be due to linkage of rust resistance and certain morphological characters. It is of interest also that, for the characters under consideration, the presence of a particular "C" chromosome does not necessarily shift the phenotype appreciably toward that of the Vulgare parent (Table 5). This is in agreement with conclusions reached earlier (10).

CONCLUSIONS

A number of *Triticum vulgare* derivatives from *T. vulgare* × *T. durum* var. Iumillo crosses have a degree of resistance to stem rust closely approximating the immunity of Iumillo. This resistance can be more readily transferred to other Vulgare wheats by hybridization than can the immunity or resistance of Iumillo itself. In spite of the considerable amount of cytological irregularity found in the material studied, a number of lines, and particularly reselections from the lines, are relatively stable 42-chromosome wheats morphologically similar to standard Vulgare types.

The effectiveness of intense selection is shown in that the rust reactions of the selected lines in the field were practically as stable as that of Iumillo. This was true notwithstanding the fact that many plants were aneuploids (having more or less than 42 chromosomes) or were heterozygous for the arrangement of one or more chromosome segments. The rigorous selection for stem rust immunity in the field had apparently eliminated all or most of the cytological irregularities involving the genes necessary for mature plant immunity.

The practical value of cytological examination of hybrid derivatives in plant breeders' material is evident.

SUMMARY

None of the various attempts to transfer to *Triticum vulgare* the immunity (complete resistance) to stem rust possessed by *T. durum* var. Iumillo has been entirely successful.

Forty-eight Vulgare-like lines of wheat and two Durum-like lines possessing some Vulgare characteristics, derived from crosses between varieties of *T. vulgare* and Iumillo by selecting plants in successive hybrid generations for stem rust resistance and Vulgare morphological characteristics, were subjected to the following tests: field tests for stem rust reaction; greenhouse tests of seedlings for their reactions to 9 physiologic races of stem rust; chromosome counts and other cytological studies; and morphological studies designed to estimate the degree of "Vulgare-ness" of lines. The lines were in F_6, F_5 or F_4 when these studies were begun.

In repeated field tests under artificially-induced epidemics of stem rust involving many physiologic races, all 50 lines were found to be immune or nearly immune in the mature plant stage in spite of the fact that most lines contained plants with abnormal chromosome numbers and behaviour.

In greenhouse tests of the reactions of seedlings, no line was immune to all of the 9 races used, but 17 Vulgare lines (derived from 5 F_2 plants) were either immune or resistant to all. The remaining 33 lines were susceptible to one or more races, but most of them were immune or resistant to a number of races. On the whole the lines appeared more resistant in advanced stages of growth than in the seedling stage.

Chromosome numbers found in plants of the forty-eight Vulgare-like lines were 38, 39, 40, 41, 42 and 43. In 5 lines only 42-chromosome plants were found; in 29 lines some, but not all of the plants examined had that number; and in 14 lines no 42-chromosome plants were found. In the two Durum-like lines all plants studied had 28 chromosomes. In all, approximately 40% of the plants examined cytologically had 42 chromosomes. Approximately 50% of the plants were found to be heterozygous for the arrangement of one or more segments of chromosomes. Eighty-one relatively stable 42-chromosome plants were found and some of these have been used to establish reselections for further breeding work.

Estimates of the degree of "Vulgare-ness" of plants based on detailed morphological studies revealed considerable differences between plants within most lines. Many plants approached the Vulgare type very closely. Combined morphological and cytological data showed that genes for the Vulgare morphological condition are not confined to the so-called C set of chromosomes.

Evidence was obtained which indicated that the mature plant immunity of one of the Vulgare-like lines when crossed with other Vulgare wheats is more simply inherited than that of Iumillo when crossed with Vulgare wheats. In both cases, however, the inheritance appears to be more complex than that of the mature plant resistance of Hope or H-44 (Vulgare derivatives of Marquis × Yaroslav Emmer) in intra-Vulgare crosses.

The aim of developing varieties of *Triticum vulgare* having the immunity to stem rust of Iumillo has been closely approximated though not fully achieved.

ACKNOWLEDGMENTS

The writers are indebted to Dr. Margaret Newton and Dr. T. Johnson of the Dominion Rust Research Laboratory for valuable advice on the interpretation of rust reactions of wheat plants and for supplying pure cultures of the various physiologic races of rust.

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STUDIES ON THE LIFE HISTORY OF THE CODLING MOTH IN SOUTHWESTERN QUEBEC[1]

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INTRODUCTION

The codling moth, a most important apple pest in some of the orchard districts of Quebec, has received the attention of this laboratory for the past three years. During that period, research work on its biology has been conducted under the direction of the Quebec Plant Protection Service.

The experimental work was carried on at Mont St-Hilaire, 25 miles south-east of Montreal; the results obtained therefore pertain especially to the southwestern part of the province of Quebec. Investigations were conducted in the insectary as well as in orchards, the insect being reared in the insectary during the entire period of its normal activities under conditions as nearly natural as possible.

The data presented in Tables 1, 2, 3, were compiled from daily records of insectary rearing in glass battery jars, the methods and apparatus used being similar to those employed by other entomologists for such studies. The experimental procedure is described in detail by Wakeland and Rice (1).

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TABLE 1.—SUMMARY OF ACTIVITY AND OVIPOSITION OF SPRING BROOD CODLING MOTHS

		1937	1938	1939
Time of moth emergence (in orchard)	First	May 30	June 1	June 8
	Maximum	June 25	July 5	July 4
	Last	July 31	July 29	July 26
Pre-oviposition period in days	Average	3.0	3.0	3.0
	Minimum	1.0	2.0	0.0
	Maximum	11.0	12.0	11.0
Number of days from moth emergence to maximum oviposition	Average	6.0	8.0	6.0
	Minimum	2.0	2.0	2.0
	Maximum	16.0	15.0	19.0
Number of days of oviposition per female moth	Average	7.0	11.0	14.0
	Minimum	3.0	2.0	5.0
	Maximum	14.0	18.0	21.0
Number of days from emergence to last oviposition	Average	11.0	15.0	17.0
	Minimum	5.0	8.0	9.0
	Maximum	24.0	23.0	22.0
Number of eggs per female moth	Average	5.6	43.9	25.8
Life of male moths in days	Average	9.0	13.0	11.0
	Minimum	1.0	2.0	1.0
	Maximum	24.0	26.0	25.0
Life of female moths in days	Average	10.0	13.0	12.0
	Minimum	2.0	2.0	1.0
	Maximum	25.0	25.0	27.0

TABLE 2.—SUMMARY OF FIRST GENERATION CODLING MOTH DEVELOPMENT IN INSECTARY

		1937	1938	1939
Time of egg deposition	First	June 10	June 8	June 8
	Maximum	June 17	July 9	July 7
	Last	July 30	Aug. 4	Aug. 23
Incubation period in days	Average	8.0	8.0	8.0
	Minimum	5.0	5.0	3.0
	Maximum	14.0	15.0	13.0
Time of hatching	First	June 19	June 18	June 21
	Maximum	July 6	July 14	July 24
	Last	Aug. 7	Aug. 4	Aug. 13
Larval feeding period in days	Average	23.0	24.0	22.0
	Minimum	16.0	14.0	16.0
	Maximum	43.0	45.0	34.0
Time of cocooning and pupation period	First	July 17	July 11	July 13
	Maximum	July 25	July 19	July 25
	Last	Sept. 1	Aug. 8	Aug. 1
Pre-pupal and pupal period in days	Average	15.0	17.0	18.0
	Minimum	10.0	10.0	13.0
	Maximum	25.0	32.0	24.0
Time of moth emergence	First	July 29	July 27	Aug. 1
	Maximum	Aug. 9	Aug. 12	Aug. 7
	Last	Aug. 20	Aug. 31	Aug. 20
Pre-oviposition period in days	Average	4.0	2.0	2.0
	Minimum	1.0	1.0	1.0
	Maximum	10.0	8.0	5.0
Number of days from moth emergence to maximum oviposition	Average	6.0	4.0	4.0
	Minimum	3.0	1.0	1.0
	Maximum	11.0	9.0	8.0
Number of days of oviposition per female moth	Average	6.0	8.0	10.0
	Minimum	1.0	1.0	1.0
	Maximum	14.0	15.0	17.0
Number of days from emergence to last oviposition	Average	11.0	10.0	12.0
	Minimum	6.0	3.0	3.0
	Maximum	19.0	16.0	17.0
Number of eggs per female moth	Average	14.6	50.4	51.3
Life of male moths in days	Average	14.0	11.0	12.0
	Minimum	2.0	3.0	3.0
	Maximum	34.0	25.0	22.0
Life of female moths in days	Average	18.0	11.0	12.0
	Minimum	2.0	3.0	2.0
	Maximum	36.0	24.0	19.0
Life cycle in days from 1st oviposition to moth emergence exclusive of pre-oviposition period	Average	46.0	49.0	48.0
	Minimum	31.0	29.0	32.0
	Maximum	82.0	92.0	71.0

To study the activities of spring brood adults, moths were secured every day in the spring from apple storage plants. By this method it was possible to collect freshly emerged moths throughout the period of spring

brood activity in the orchard, as determined by means of bait-traps. The data are included in Table 1. It was necessary to collect moths from storage plants because no success was obtained in keeping enough larvae alive over winter to secure a sufficient number of adults for rearing work in the spring. It is difficult to say to what extent this way of securing moths has affected the data presented in Table 1. However, in general, they seem to range within a fair limit of correctness.

The figures under "number of eggs per female moth" in Tables 1 and 2, in 1937, include only eggs laid on leaves, whereas those of 1938 and 1939 include also the eggs laid on the sides and bottoms of the rearing jars. It should also be noted, in Table 2, that the last eggs laid in 1938 and 1939 failed to hatch because they were accidentally injured. As will be seen later in this paper, it should also be mentioned that in 1938 and 1939 the percentage of larvae which pupated was very low and most of them were early maturing larvae. This explains why maximum pupation for those years comes shortly after the maximum hatching date (Table 2). Part of the larvae of the first generation reared in the insectary transformed to produce a partial second generation as follows:—

Year	Number of larvae	Number of moths emerging	Larvae transforming
			%
1937	543	269	49.5
1938	818	214	26.2
1939	319	58	18.2

TABLE 3.—SUMMARY OF SECOND GENERATION CODLING MOTH DEVELOPMENT IN INSECTARY

		1937	1938	1939
Time of egg deposition	First	Aug. 2	July 29	Aug. 5
	Maximum	Aug. 7	Aug. 15	Aug. 18
	Last	Sept. 5	Sept. 2	Aug. 31
Incubation period in days	Average	7.0	8.0	6.0
	Minimum	5.0	6.0	5.0
	Maximum	8.0	11.0	8.0
Time of hatching	First	Aug. 9	Aug. 5	Aug. 17
	Maximum	Aug. 16	Aug. 15	Aug. 26
	Last	Aug. 29	Aug. 31	Aug. 29
Larval feeding period in days	Average	24.0	29.0	26.0
	Minimum	21.0	20.0	20.0
	Maximum	27.0	36.0	31.0

The maximum larval feeding period as given in Table 3 is somewhat short, because the last larvae to leave the fruits could not be included, as they came out in October after work had been terminated for the season. Table 3 does not show the important fact that, in 1939, doubtless as a result of the prevailing unfavourable weather, few eggs were laid in the cages and especially on the apple leaves used in the latter. The relative numbers of eggs laid on leaves and on the walls of the cages are given below:—

—	1937	1938	1939
Eggs on leaves	2465	2329	124
Eggs on cages	No count	3419	1519
Total eggs		5748	1643

ORCHARD STUDIES

In the orchard the most important work was the study of the time of moth activity by means of bait-traps. The traps were used at the rate of 10 per acre of orchard. In 1937, eight traps were operated in a small neglected orchard containing several varieties of apples. In 1938, an equal number of traps was used in the same orchard, and 10 others were hung in a block of McIntosh in a neighbouring orchard where no special sprays were applied against the codling moth. In 1939, 18 traps were hung in the latter orchard.

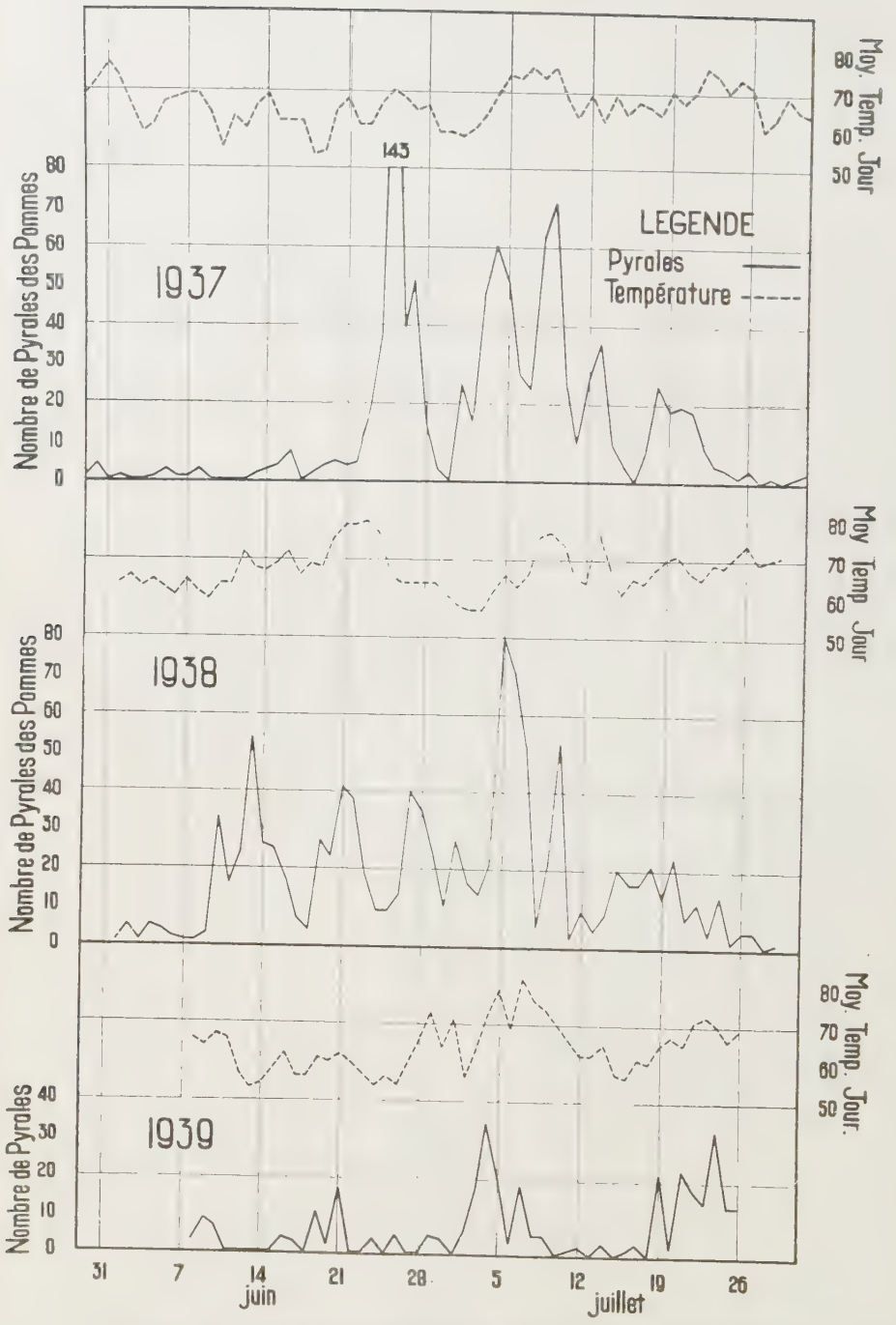
The type of trap used consisted of a white enamelled pudding dish of an approximate capacity of one quart. A hanger was made of 3 wires attached through small holes equidistant about the rim of the dish; the other ends of the wires were tied together to make a hook. The traps were suspended from a cross-arm supported on a pole located in an opening of the tree as close to the centre as possible, and from 2 to 3 feet below the top of the highest limbs. The traps were raised and lowered by means of a cord running through a pulley fixed to the cross-arm.

The traps were examined every day to remove captured insects, to record the numbers of codling moths, and to maintain the bait at its original quantity. The traps were cleaned and the bait changed twice a week or after heavy rains.

The bait used was a mixture of 1 part of molasses and 19 parts of water to which was added a small piece of fresh yeast to obtain better fermentation, and was prepared about half a day before being put in the traps. In order to make the bait more attractive a small vial filled with pine tar and attached to a square cork float was placed in each trap. This procedure was discarded in 1938, because the vials sank when the cork became too heavy from absorbed water.

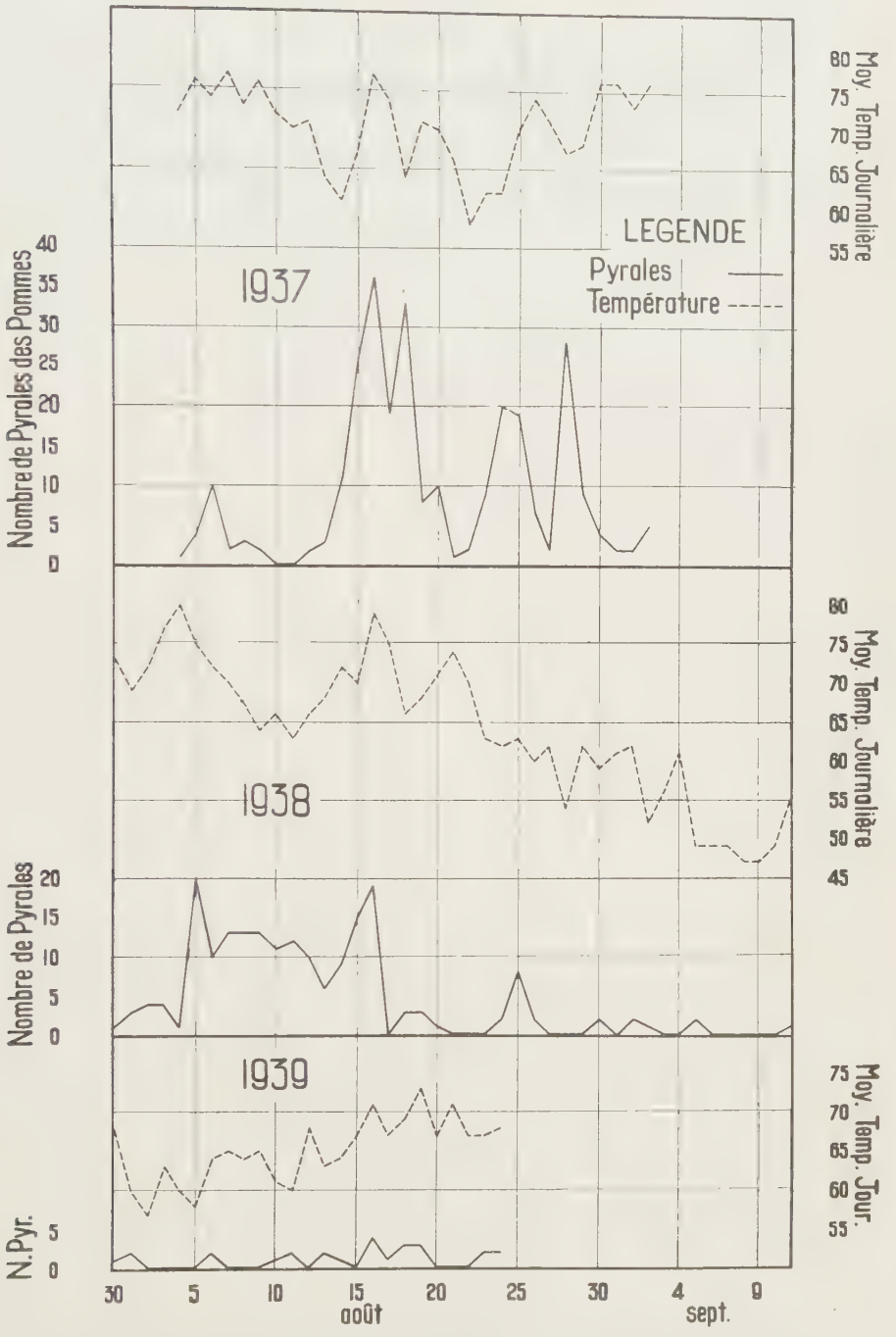
The daily catches of codling moths in bait-traps are shown graphically in Figures 1 and 2. It will be observed that in 1937 spring brood moth activity was mostly concentrated between June 25 and July 19 with the highest peak on June 25. The first brood for the same year was active

FIGURE 1



CAPTURES DE PYRALES DES POMMES DANS LES PIEGES-APPATS
Pyrales du Printemps
1937–1938–1939

FIGURE 2



CAPTURES DE PYRALES DES POMMES DANS LES PIEGES-APPATS
1ère génération
1937–1938–1939

between August 14 and 28. In 1938, the flight of the spring and the first brood moths attained important proportions respectively 16 and 10 days earlier than in 1937. In spite of the fact that heavy first brood flight occurred earlier than in 1937, it lasted about 10 days later in the summer. The shortest flight periods of both spring and first brood moths were in 1939. In that year, moths were not active in the spring until June 8, which was 8 to 10 days later than the two previous years. Moths of the first generation were not captured after August 23, which was 10 days and 14 days earlier than in 1937 and 1938 respectively. In 1939 the moth population, as shown in Table 4, was the smallest for the three years recorded.

Figures 1 and 2 show that the moths were active when the average daily temperature was above 60° F., and the most important peaks of flight occurred when the temperature was at or above 65° F.

TABLE 4.—TOTAL CAPTURES OF CODLING MOTHS IN BAIT-TRAPS IN 1937–38–39

Month	Number of days			Number of moths			Number of: Males			Number of: Females		
	1937	1938	1939	1937	1938	1939	1937	1938	1939	1937	1938	1939
Spring brood												
May	3	0	0	5	0	0	2	0	0	3	0	0
June	30	30	23	356	516	74	199	328	45	157	188	29
July	31	31	26	602	534	252	327	347	118	275	187	134
Total:	64	61	49	963	1050	326	528	675	163	435	375	163
First brood												
July	0	0	1	0	0	1	0	0	0	0	0	1
August	31	31	24	273	185	25	138	103	15	135	82	10
September	2	11	0	7	6	0	2	3	0	5	3	0
Total:	33	42	25	280	191	26	140	106	15	140	85	11
Total, both broods:	97	103	74	1243	1241	352	668	781	178	575	460	174

TABLE 5.—PERCENTAGES OF MALE AND FEMALE CODLING MOTHS TAKEN IN BAIT-TRAPS

	Males			Females		
	1937	1938	1939	1937	1938	1939
Spring brood	54.8	64.3	50.0	45.2	35.7	50.0
First brood	50.0	55.5	57.7	50.0	44.5	42.3
Both broods	53.7	62.9	50.6	46.3	37.1	49.4

MOTH EMERGENCE IN ORCHARD, CAGES, AND INSECTARY

The data in Table 6 present in comparison the emergence of first brood moths as recorded in the orchards by means of bait-traps, in outside cages, and in the insectary, for the years 1937 to 1939 inclusive.

The outside cages were of temporary construction, 10 × 6 × 6 inches, with the top, bottom and frame of softwood and the 4 open sides covered with cotton fly muslin. They were hung under apple trees, and larvae collected in orchards by means of corrugated paper bands were placed in them. Fifty of these bands, 4 inches wide, were placed around well scraped

TABLE 6.—EMERGENCE OF FIRST BROOD CODLING MOTHS

	Orchard			Cages			Insectary		
	1937	1938	1939	1937	1938	1939	1937	1938	1939
First	Aug. 4	July 31	July 31	July 20	July 30	Aug. 10	July 30	July 27	Aug. 1
Maximum	Aug.16–18	Aug.5–16	Aug. 16	Aug. 10	Aug. 20	Aug. 14	Aug. 3–9	Aug. 3–12	Aug. 7
Last	Sept. 2	Sept. 11	Aug. 24	Aug. 20	Aug. 31	Aug. 22	Aug. 18	Aug. 31	Aug. 20

trunks of apple trees, in order to catch the full-grown larvae which were searching for places to spin their cocoons. As far as possible the larvae were removed from the bands at regular intervals of 4 to 6 days commencing in the latter part of July, each lot of larvae being put in a separate cage.

CONCLUSIONS

There was a gradual decrease of the partial second generation from 1937 to 1939, which is in close relationship with a similar decrease of the mean daily temperature over these years. It is evident that the development of the codling moth depends a great deal upon weather conditions, especially temperature. There is, however, no doubt as to the existence of a partial second generation of codling moth in Quebec, at least in the southwestern districts.

It would seem, from the data presented, that the second generation would be important only in certain years. For this reason the studies should be continued in order to secure more representative data and to check upon those already obtained.

In the meantime, the information already secured may be useful for the timing of spray applications for the control of codling moth in southwestern Quebec.

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THE RELATIONSHIP OF WEEDS TO LOSSES CAUSED BY COMMON ROOTROT IN WHEAT[1]

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It has been observed, when cereal crops are stunted and thinned by rootrotting organisms, that weeds may develop abundantly. Russell (7) in discussing losses due to take-all (*Ophiobolus graminis* Sacc.) states, "When wheat plants are weakened by the fungus the weeds which are present make greater headway and offer keener competition than they do when the wheat plants are free from the disease". Likewise browning rootrot due to *Pythium* spp. (1) retards the wheat crop, and frequently prevents normal tillering and allows weeds to become well established. While no observations have been recorded so far as the writer is aware of similar conditions with common rootrot (*Helminthosporium sativum* Pamm. King and Bakke and *Fusarium* spp.) it is probable that a severe form of the disease will reduce the competitive power of wheat and give weeds a chance to develop.

Experiments have been conducted on summerfallowed soil at Rosthern, Saskatchewan, during the three years 1937 to 1939, to test the effect of inoculation of Reward wheat with *Helminthosporium sativum*, on the development of other plants growing in competition with the crop.

METHODS

Inoculations of wheat with *Helminthosporium sativum* were made by treating the seed prior to sowing with a sporulating culture of the fungus grown on oat hulls for about one month. Inoculation was accomplished by moistening the seed with 10% of its weight of water and adding 5% of its weight of sifted oat-hull culture. Following thorough shaking to give a uniform coating of inoculum, the seed was air-dried for a few hours, and coarse pieces of the oat-hull medium were sifted out.

EXPERIMENTS

The 1937 experiment consisted of four blocks arranged in a row, each of which contained five plots. Five treatments were assigned to the plots of each block at random. Each plot was 3 rods long and 8 feet wide. The treatments were: wheat; wheat, inoculated with *Helminthosporium sativum*; wheat and flax; and wheat, inoculated with *H. sativum*, and flax. In addition, a fifth plot in each block was seeded to flax alone. This treatment was randomized with the others though not entering into the statistical analysis, due to the absence of wheat. Flax was used in the experiment to offer competition to the wheat for moisture, in case the naturally occurring weeds were not uniformly and thickly distributed throughout the plots.

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The wheat was seeded at a rate of 5 pecks and the flax at 20 pounds per acre. The wheat was sown first at a depth of about 2 inches and flax was then seeded at a depth of about 1 inch.

At harvest time two areas of 1 square yard, taken at random, were harvested from each plot by pulling the plants. The plants were separated into three groups, namely: wheat, flax and weeds. They were then air-dried for about three weeks and weighed. The yields taken in grams were converted to tons per acre. These are shown in Table 1.

TABLE 1.—AIR-DRIED WEIGHTS IN TONS PER ACRE

Crop mixture	Treatment	Wheat	Weeds	Flax	Total
Wheat	Uninoculated	1.024	0.086	—	1.110
Wheat and flax	Uninoculated	0.999	0.055	0.170	1.224
Wheat	Inoculated	0.826	0.549	—	1.375
Wheat and flax	Inoculated	0.636	0.238	0.552	1.426
Flax	None	—	0.387	0.885	1.272

The data for wheat yields and weed yields were submitted to analyses of variance and the mean squares are given in Table 2.

TABLE 2.—ANALYSES OF VARIANCE OF DATA SUMMARIZED IN TABLE 1

Source of variance	Degrees of freedom	Mean squares*	
		Wheat yields	Weed yields
Blocks	3	1438	0.00909
Treatments	3	**18141**	**0.20411**
Inoculation	1	**44100**	**0.41699**
Flax	1	6480	**0.11679**
Inoculation × Flax	1	3844	**0.07854**
Error	9	1511	0.00805

* Mean squares in bold face type exceed the one per cent level of significance.

Inoculation with *Helminthosporium sativum* reduced the yield of wheat and increased the yield of weeds significantly. Competition from flax, and the interaction, inoculation × flax, had the effect of reducing the yields of both wheat and weeds, the latter significantly. The reduction in stand and vigour of the wheat plants due to inoculation appears to have allowed the flax plants to compete successfully.

The design of the 1938 and 1939 experiments was based on a three factor experiment with each factor at two levels, as described by Yates (10). The competition element was provided by seeding certain plots broadcast with a mixture of common annual weeds, after the wheat had been seeded 2 inches deep, with a V-belt, rod-row seeder. The weeds used were lamb's quarter (*Chenopodium album* L.) 34 grams, and redroot pigweed (*Amaranthus retroflexus* L.) 16 grams per plot. The weed seeds were lightly raked into the top soil. The remaining plots also were raked in a like manner,

and kept free from volunteer weed plants. Half the plots were seeded at a rate of one bushel of wheat per acre, and the others at double that rate. The third factor was inoculation of the seed with *Helminthosporium sativum* as opposed to no inoculation. Combining these in all possible ways, there are eight treatments, as listed in Table 3. These treatments were replicated four times in eight blocks, confounding the second order interaction effect with blocks.

Each plot was of 9 rows, 6 inches apart and $\frac{1}{2}$ rod long. At harvest the centre five rows were harvested after cutting off 11 inches from each end. The harvested area was, thus, 16 square feet. In 1938 the data taken included yield of dry matter for pulled wheat and weed plants and the yield of grain. In 1939 only the yield of grain was taken. These sets of data are presented in Table 3, and analyses of variance in Table 4.

Table 3. Yield from the 1938 and 1939 experiments in tons and bushels per acre

Treatments	Air-dried weights 1938		Yield of grain	
	Wheat	Weeds	1938	1939
	Tons	Tons	Bushels	Bushels
None (control)	1.108	0	10.33	56.55
Inoculation	1.090	0	13.70	44.57
Weeds	1.280	0.391	12.15	47.40
Inoculation and weeds	0.547	1.303	5.62	45.10
Double rate of seeding	1.149	0	7.35	54.20
Double rate and inoculation	1.242	0	14.67	49.67
Double rate and weeds	1.017	0.120	5.30	50.97
Double rate, inoculation and weeds	0.735	1.078	7.92	43.95

Table 4.—Main and interaction effects of treatments as determined by Yates' method (10) in tons and bushels per acre

Treatment effects	Dry weight of wheat plants 1938*	Yields of grain*	
		1938	1939
	Tons	Bushels	Bushels
Inoculation	**−0.235**	+1.70	**−6.46**
Weeds	**−0.252**	**−3.76**	**−4.39**
Double rate of seeding	+0.029	−1.64	+1.29
Inoculation and weeds	**−0.272**	**−3.65**	+1.80
Double rate and inoculation	*+0.141*	**+3.27**	+0.68
Double rate and weeds	−0.067	−0.64	−0.08

* Data in bold face type and italics exceed the one per cent and five per cent levels of significance respectively.

In 1938 there were significant reductions in dry plant weights of wheat due to inoculation, to weeds and to the interaction effect between the two. The yield of threshed grain, however, in the same year showed a non-significant increase due to inoculation. This apparent anomaly has a logical explanation on the basis of rainfall. Figure 1 shows the rainfall to be very small during June, and while the July rainfall was normal, the greater portion of it fell about the middle of the month. During the excessive

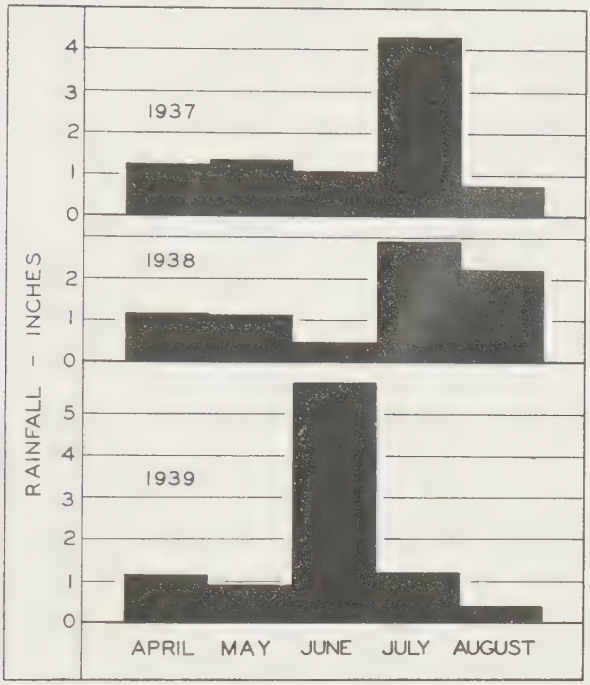

FIGURE 1.—Rainfall by months for the crop seasons of 1937 to 1939 at Rosthern, Saskatchewan.

drought to the middle of July the wheat in uninoculated plots grew very poorly and came to early maturity, being unable to utilize the rainfall when it came. At the same time the wheat of the inoculated plots that were free from weeds, due to being thinned greatly in stand was still growing fairly well and was able to utilize the rainfall in filling the kernels, and consequently yielded more than the controls.

In 1939 both inoculation and weeds significantly reduced yields of wheat. Unlike the 1938 result there was no significant interaction effect between the two treatments on yield. Here again the explanation must be sought in the rainfall data, Figure 1. During May, the rainfall was subnormal, being less than 1 inch. Rain fell on 15 days during the month and except on the first day did not reach 0.1 inches in any one day. Thus the distribution of May rainfall was not favourable to the germination of weed seeds, sown in dry top soil. The weeds germinated well in June after the heavy rains began. Meanwhile the wheat, seeded into moist soil, germinated and grew normally. The inoculated wheat recovered before weeds were present to take advantage of the initial stunted growth due to disease.

DISCUSSION

Pavlychenko and Harrington (6) have shown that many noxious weeds make relatively small growth during the early stage of development as compared with the cereal crops. When growing in competition with

cereals, these weeds may be largely smothered, some cereals being very effective competitors and others, such as wheat and flax being less effective. When competition was reduced by wide spacing of rows, many weeds outyielded the cereals by wide margins.

Inoculation with *Helminthosporium sativum* has been shown (8) to result in stunting of the plants. Under good conditions for growth most surviving plants recover from the initial effects and may produce almost a normal yield of grain. If, however, weeds have started at the same time that the wheat germinated they will benefit by the reduced competition from the diseased wheat seedlings. When the wheat plants pass the initial stunting phase, their full recovery is prevented by the competition of the weeds for soil moisture, nutrients and light. There is, thus, an interaction effect between the disease and weeds resulting in a decreased yield of grain.

The time element in the interaction is important. If for some reason the germination of weed seeds is delayed as in the 1939 experiment, the diseased plants may have recovered in part from the initial stunting. In consequence the weeds may not gain much advantage due to the disease.

It appears that any practice that controls or eradicates weeds may be a valuable aid in minimizing losses due to common rootrot as well as other rootrots, such as take-all and browning. Likewise any practice tending to reduce the effects of rootrot on the crop may be of value in the suppression of weeds by competition.

There is a suggestion in the 1937 experiment that might be worth some study. The losses in wheat yields caused by *Helminthosporium sativum* may be made up in part by seeding with the wheat a second crop such as flax, which is not susceptible to the same disease. The practice of sowing wheat and flax together has been tried experimentally in Western Canada, but has not been adopted as a standard cropping method. It has been found that flax does not ordinarily compete with wheat sufficiently well under dry-land conditions (2, 3, 4, 5, 9) to justify the expense of separating the seed. There may, however, be a place for the practice in certain fields where the farm operator has good reason to believe that one or more of the rootrots may cause serious injury. In the case of browning rootrot of cereals the practice may not prove useful since a practicable method of control exists in the use of phosphatic fertilizers. Similarly, take-all can be effectively controlled by a proper rotation.

SUMMARY

Helminthosporium sativum, a principal cause of common rootrot of cereals in Western Canada, may retard wheat in the seedling stage so that weeds become well established. The weeds may then prevent the normal tendency of the crop to recover, resulting in reduced yields of grain.

ACKNOWLEDGMENT

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A BACTERIAL DISCOLORATION OF PRINT BUTTER[1]

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A number of cases of an unusual surface discoloration of print butter have been studied in this Division during the past few years. This defect has caused not only considerable trouble and annoyance, but also financial loss to butter jobbers and retailers, as in two cases carlots of butter were involved. The discoloration was observed on the surface of print butter from creameries in widely separated localities in the Prairie Provinces and in Ontario. The defect was not apparent in the butter as it came from storage in 56-pound boxes, but appeared only after the butter had been cut into prints and held for 5 to 9 days at temperatures usually found in retail store refrigerators (40°–55° F.). The butter was of first grade quality and some lots were 40 score in flavour. In all cases the butter was mildly salted, containing between 0.5 and 1.0% salt, but in other respects its composition was normal as shown by the usual methods of analysis.

Hiscox (1) reported a similar defect found in mildly salted butter (0.3–0.55% salt) which was cut into "pats" and held in cold store awaiting distribution. The butter had previously been carried in ships' refrigerators during transportation to the British market. Two strains of bacteria producing a bluish black pigment were isolated from the discoloured areas of the butter. These strains differed somewhat in colony formation and vigour of growth, but in their general characteristics appeared to be the same.

MATERIALS AND METHODS

Description of the Defect

In the samples submitted to this Division the defect was evident as a black to reddish brown discoloration in irregular patches of varying size on the surface of the butter print, as shown in Figure 1. The discoloration was found to penetrate to a depth of approximately one-eighth inch. It did not appear to spread on further holding of the prints, and there was no evidence of discoloration on freshly cut surfaces of the interior of the butter at 40° F.

Bacteriological Examinations of Defective Butter

Microscopic examination of stained preparations of the discoloured areas of the defective butter regularly revealed large numbers of small Gram negative rods. Plate counts of the discoloured surface areas and of the normal butter from the interior of the print were made on beef infusion agar plus 1% lactose at 25° C. and on nutrient agar plus 1.5% salt (NaCl) at 15° C. In every instance the counts from the surface of the butter were much higher than from the interior. The plate counts of two typical samples of discoloured prints are shown in Table 1.

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FIGURE 1.—A typical case of discoloration of print butter caused by *Ps. nigrifaciens.*

TABLE 1.—SURFACE AND INTERIOR COUNTS OF DISCOLOURED BUTTER

Sample No.	Medium	Bacteria per ml.	
		Discoloured surface butter	Interior butter
I	Beef infusion at 25° C.	630,000	23,000
	Nutrient agar + 1.5% NaCl at 15° C.	1,000,000	26,000
II	Beef infusion at 25° C.	97,000,000	2,050,000
	Nutrient agar + 1.5% NaCl at 15° C.	45,500,000	1,770,000

An organism capable of causing the defect was isolated by plating from defective areas on nutrient agar plus 1.5% salt (NaCl) and by streaking small portions on the surface of slopes of the same medium. Incubation of both plates and slopes was at 4° C. and 15° C. After 5 to 9 days a blackish growth developed on the slopes at both temperatures and small brownish to blackish colonies on heavily seeded plates. On plates of higher dilutions, where colonies were well separated, no pigmented colonies were visible. On replating the defective butter, using water blanks containing 1.5% salt, a few well isolated pigmented colonies were usually obtained at temperatures of 4° and 15° C. after 4 to 5 days. From both plates and slopes a number of cultures producing black colonies on nutrient agar plus 1.5% NaCl were isolated.

More recent samples of butter showing typical discoloration were examined by the Burri smear technic suggested by Long and Hammer (2) wherein tiny portions of butter are smeared on agar slopes. Well separated pigmented colonies were easily obtained with this method from both discoloured and normal surface areas of the butter prints and from the interior of the butter where no discoloration had developed. In a test to note the distribution of the pigment-producing organisms and their relative incidence, it was found that from discoloured surface areas 50% of Burri smear slopes contained pigmented colonies, and from normal surface and interior butters 74 and 94% respectively. From 10 to 90% of the colonies appearing on individual slopes were pigmented. In one case pigment-producing organisms were isolated from butter prints showing no discoloration but which were from the same churning as prints which had developed the defect.

The organism was capable of remaining viable in butter over a considerable period of time, and was isolated from defective prints after nine months' storage at 4° C.

Reproducing the Defect in Butter

The defect was reproduced readily by inoculating butter granules with a small amount of an aqueous suspension of the organisms during the working process when salted at the rate of 0.5% (equivalent to a concentration of approximately 3% salt in the serum) and held at temperatures of 4° C. and 15° C. Washing the butter granules with water infected with the organism also reproduced the defect. When the butter was unsalted or when salted at the rate of 1 and 2% (a salt concentration of 6% or higher in the butter serum) the defect was not reproduced even after prolonged holding at 4° C.

Butter from well pasteurized sweet cream inoculated with the organism on the previous night or just before churning also developed the defect when it was salted at the rate of 0.5% and held at a temperature of about 5° C.

The discoloration in these experimental butters was usually discernible after 5 to 9 days and appeared as small blackish spots or as larger irregular brownish areas on the surface. When new surfaces of the experimental butters were cut, the discoloration appeared on the fresh surfaces. The same conditions of temperature and salt content which were favourable to the development of the defect in commercial butter also demonstrated the discoloration in experimental butter.

DESCRIPTION AND REACTIONS OF THE ORGANISM

The following description of the organism is based on detailed studies of a number of cultures isolated from several samples of discoloured butter. All media contained 1.5% salt (NaCl) unless otherwise stated.

Morphology:

Form: Rods with rounded ends; on nutrient agar slopes after 2 days at 4° C. and 1 day at 21° C. cells varied from 0.3 to 0.7 by 1 to 5 microns, with most cells about 0.5 by 1.5 to 2 microns.

Arrangement: Usually singly or in pairs; quite long chains observed in old viscous milk and broth cultures.

Motility: Actively motile, with one polar flagellum. With some preparations fine threads of a gelatinous nature appear as though attached to the cells, and should not be confused with the flagella proper.

Staining: Gram negative; stains readily with the usual stains.

Spore formation: Spores not observed; the organism easily destroyed by heat.

Capsules: Not found, but in some cultures the cells appear to be embedded in a gelatinous material.

Cultural Characteristics:

Agar Slope: Growth filiform, smooth, moist and glistening with blackish pigmentation well developed after 48 hours at 4° C. and 15° C. The growth appears slightly fluorescent in the early stages. In old cultures growth slightly viscid and the pigment appears to fade to a dirty greyish colour. A brownish colour diffuses through the medium under the growth.

Agar Stab: Blackish pigmented growth appears on the surface and to a depth of about ½ inch at 4° C. and 15° C. Meagre non-pigmented growth occurs along the line of inoculation.

Agar Colony: Surface colonies convex, smooth, glistening, entire, round, and 2 to 4 mm. in diameter. The colonies appear slightly fluorescent in the early stages; pigmentation evident after 2 to 5 days at 4° C. and 15° C. A brownish colour diffuses through the medium with heavily seeded plates. Pigmentation less intense at 15° C. than at 4° C. Subsurface colonies ellipsoidal and non-pigmented.

Gelatin Stab: Slight liquefaction and pigmented surface growth apparent after 24 hours at 15° C. and after 48 hours at 4° C. Early liquefaction is crateriform, changing later to saccate. Slight liquefaction proceeds slowly without NaCl but growth is non-pigmented.

Potato Dextrose Agar: No growth even with NaCl.

Potato Slope: No growth even with NaCl.

Nutrient Broth: Turbidity evident after 24 hours at 15° C. but slower at 4° C. After 5 to 6 days a black pigmented ring and then a pellicle forms at the surface, and later a black sediment. The broth turns a brownish tea colour with age. The surface growth becomes quite viscid and stringy in old cultures. The pH changes from 6.8 to about 8.2 or 8.4.

Litmus Milk: A slight blackish pigmented ring visible at the surface after 3 days at 15° C. and somewhat later at 4° C. After about 6 days a blackish pellicle forms. Reduction of the litmus from the top down occurs with an alkaline reaction later, but no coagulation. Some digestion after 48 to 72 hours which develops progressively with practically total digestion after 6 weeks at both 4° C. and 15° C. A putrid odour develops with the digestion and is evident after 48 hours. In old cultures the surface growth becomes viscous. The characteristic changes take place without addition of NaCl but much more slowly than when NaCl is present.

Cream (20% fat): Pigmentation and a putrid odour develop at 4° C. and 15° C. with NaCl added, but digestion not apparent. Without NaCl some pigmentation develops slowly at both 4° C. and 15° C. after 3 to 4 weeks.

Biochemical Characteristics:

Gas production: Not observed in any media.

Indol: Negative after 72 hours at 4° C. or 15° C.

Nitrates: Not reduced after 7 days at 4° C. or 15° C.

Ammonia: Positive in nutrient broth after 12 days at 4° C.

Starch: Hydrolyzed at 4° C., 15° C. and 25° C., most rapidly at the higher temperatures.

Reaction change: An alkaline reaction produced in sucrose, maltose, lactose, dextrose, mannite and raffinose broths. A black pigmented ring and pellicle form as in plain broth, and the pH changes from about 6.8 to approximately 8.2.

Natural fats: not hydrolyzed.

Growth Conditions:

Temperature relation: Optimum temperature about 25° C. but growth non-pigmented. At both 4° C. and 15° C. pigment is produced but growth develops more slowly than at 25° C. At 15° C. growth is more rapid than at 4° C. but pigmentation is less intense. Scanty non-pigmented growth develops slowly at 33° C.–35° C. but at 37° C. no growth is evident.

Oxygen requirements: Aerobic. No growth observed in shake cultures except at the surface, nor with the pyrogallic acid technic. No growth observed under cover slips placed on streaked agar plates.

pH of medium: Good growth and pigmentation take place at pH 6.8, 7.4 and 8.4 at 4° C. No apparent growth occurs at pH 5.2 in nutrient broth or on agar slopes.

Growth on Culture Media

The organism was not able to grow on the usual cultural media in the absence of salt, but good growth was established on nutrient agar, tryptone glucose agar, nutritive caseinate agar, nutrient gelatin, and in nutrient and tryptone broths when 1.5% salt was added. On fresh beef infusion agar the organism was able to grow without the addition of salt, but growth was non-pigmented. A 4% casein agar plus 1.5% salt, and butter serum diluted to contain about 2% salt were also very satisfactory culture media. However, on potato dextrose agar and fresh potato slants no growth was

observed even in the presence of 1.5% salt, and on corn meal agar plus 1.5% salt only scanty growth developed slowly. The nutrients available in culture media of vegetable origin did not appear suitable for the growth of the organism.

Pigmentation by the Organism

Temperature was found to be an important factor in the formation of pigment by the organism when cultured on media containing 1 to 3% salt. Pigmentation occurred at temperatures of 4° C. and 15° C. but was more intense at the lower temperature. At 25° C. or higher the production of pigment was inhibited. With pigmentation at the lower temperatures a brownish colour diffused through the medium especially when growth was heavy.

The permanence of the pigment-producing character of the organism was tested by carrying a number of cultures through 10 to 12 transfers under adverse conditions, viz., on nutrient agar containing 5% salt (NaCl) at 25° C. Transfers were made every 2 or 3 days, and under these conditions good growth was obtained but at no time was pigment observed. However, when the cultures were transferred back to nutrient agar with 1.5% salt and incubated at 4° C. the characteristic pigmentation reappeared.

The solubility of the pigment was studied by washing the pigmented growth from 1.5% salt agar slopes with various solvents. In cold solution the pigment was found to be insoluble in water, 1% brine, ether, chloroform, acetone, 95% and absolute ethyl alcohol, 10% sodium hydroxide, and formalin. Hiscox (1) found the pigment of the organisms studied by her to be soluble in formalin to a bright blue solution, and also to give unstable solutions in potassium and ammonium hydroxide, but that it was insoluble in many of the usual solvents.

The Influence of Salt (NaCl)

Salt not only was found to be essential for the proper growth of the organism, but the concentration definitely influenced its growth and pigmentation. Cultures of the organism were grown at 4° C. in nutrient broth and on nutrient agar slopes containing 0.0, 0.5, 1.0, 1.5, 2.5, 5.0, 7.5, and 10.0% NaCl. No growth was obtained in the absence of salt, while maximum growth and pigmentation appeared with 1.5 and 2.5% salt. At a salt concentration of 5.0% or higher, pigmentation was absent, and growth was greatly decreased with salt concentrations of 7.5 and 10.0%. No growth was apparent on slopes containing 10.0% salt after 8 days, but scanty growth was present after 4 weeks.

While the organism was unable to grow in a salt-free medium, cultures remained viable for a number of days. When salt-free tryptone broth was inoculated with the organism and incubated at 4° C. for 7 days, no growth was observed, but with the addition of a sterile salt (NaCl) solution to the incubated tubes to give concentrations of approximately 1%, good growth and characteristic pigmentation were present after 4 days at 4° C.

The addition of NaCl as such did not appear essential as the organism grew slowly in milk and nutrient gelatin. In milk the characteristic changes took place but in gelatin no pigmentation was apparent. The addition of salt to the above media, however, greatly hastened growth and pigment production.

The influence on growth and pigment production of salts other than NaCl was tried by the addition of 1.5% potassium chloride (KCl), potassium iodide (KI), and calcium chloride ($CaCl_2$) to nutrient agar. With KCl good growth and pigmentation were obtained after 6 days at 4° C., while KI gave good growth but slight pigmentation. With $CaCl_2$, however, growth was scanty and pigmentation absent. None of the salts gave as marked growth and pigmentation as NaCl.

The Effect of Pasteurization

The resistance of the organism to pasteurization was determined by inoculating tubes of sterile milk, cream and nutrient broth plus 1.5% salt, heating in a water bath to a temperature of 77° C. to 78° C. for 10 minutes, and cooling to 15° C. as rapidly as possible. The tubes were incubated at 15° C. for 12 days. No growth was apparent in any of the heated tubes but good growth and pigmentation developed in duplicate unheated tubes. When the pasteurized material was streaked on salt agar slopes no growth took place, while material from unheated tubes gave characteristic growth and pigmentation. It was evident that the minimum pasteurizing temperature of 170° F. for 10 minutes, as used in Canadian creameries, was sufficient to destroy the organism.

Identification of the Organism

The characteristics of the organism described herein are in the main the same as those of the pigment-producing organisms isolated from discoloured butter and described by Hiscox (1). From their general characteristics Hiscox regarded the organisms isolated by her to belong to the genus *Pseudomonas*, although special characters did not agree closely with those of any similar organism described. At the time Hiscox (1) did not regard the organism as a new species and did not assign a specific name to it.

However, as the organism is considered to be an unnamed species, the name *Pseudomonas nigrifaciens* is suggested for it.

The Distribution of the Organism

The discoloration of print butter caused by the pigment-producing organism has been noted by Hiscox (1) in butter on the British Market from overseas Dominions. The author has found it in butter[1] marketed in St. Louis, Mo., U.S.A., and in butter manufactured in several creameries in the Provinces of Saskatchewan, Manitoba and Ontario. The occurrence of the defect in such widely separated areas indicates that the organism is widely distributed in nature, and that the defect may occur occasionally in butter even of the highest market quality when salt and temperature conditions are favourable.

SUGGESTED METHODS OF CONTROL

The discoloration of the surface of mildly salted butter by *Pseudomonas nigrifaciens* again indicates the importance of micro-organisms as a cause of unusual defects in this product. Modern procedures in butter manufacture tend to reduce the total number of bacteria present and to produce

[1] The author had the opportunity of examining and studying several quarter-pound prints of discoloured butter which had been sent to the Dairy Laboratory of the Iowa Agricultural Experiment Station for analysis. The butter was mildly salted and had been in the retail trade for some weeks. Organisms producing the characteristic blackish pigment were readily isolated from the defective butter.

a butter of nearly neutral pH. Such conditions are especially favourable to the growth of certain species of bacteria which may occasionally find their way into the product after pasteurization. The low heat resistance of the organism described suggests that recontamination had occurred after pasteurization, and emphasizes the importance of thorough sanitation of all equipment as a means of control.

This particular defect is of special interest to those butter manufacturers and distributors who are marketing a mildly salted butter of high quality for a special market, as the causative organism produces the discoloration only in butter usually containing less than 1% of salt. The relationship of the growth and pigment-producing characters of the organism to salt concentration indicates that the incorporation of 1.25% of salt or more, to give a salt concentration in the serum of 7 % or higher, will effectively control the defect.

Although temperature was found to be an important factor in the production of the colour defect by the organism, this fact cannot be used as a means of control under commercial conditions. The optimum temperature range for pigment production is that usually found in retail store refrigerators. Temperatures at which pigment production is inhibited (25° C. or above) are much too high for butter storage, while temperatures low enough to control growth of the organism are not practical in most retail establishments.

While no data are available at present as to the original sources of the organism, its general characteristics place it in the group of soil or water bacteria. This fact suggests that the butter wash-water may be an important source of the organism in the recontamination of the cream or butter. Again it may gain entrance through fine soil dust recontaminating the equipment or the cream itself after pasteurization. Proper treatment of the water supplies and a thorough rinsing or sterilization of all equipment before use would help to eliminate possible trouble from these sources.

SUMMARY

Several cases of an unusual discoloration which developed on the surface of mildly salted print butter during retail distribution have been studied. An organism was isolated from the defective butter which produced a blackish insoluble pigment on culture media at temperatures of 4° C. and 15° C. with low concentrations of salt. Optimum conditions for pigmentation on culture media appeared to be at a temperature of 4° C. with salt concentrations of 1.5% to 2.5%. The defect was reproduced in butter by inoculating the cream used for churning, and by inoculating the butter directly with wash-water infected with the organism, or during the working process when 0.5% salt was added to give a salt concentration of about 3% in the serum. Experimental butters, when unsalted or containing over 1% salt to give a salt concentration of over 6% in the serum, did not develop the discoloration. Low concentrations of salt appeared to be essential for proper growth and characteristic pigmentation by the organism, but at salt concentrations of over 5% in both culture media and butter serum, pigmentation was inhibited and growth retarded.

The causal organism is described and the name *Pseudomonas nigrifaciens* is suggested for it. Under practical conditions control measures should include thorough sanitation in the plant and salting the butter with at least 1.25% salt to give a concentration of approximately 7% in the serum.

REFERENCES

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